STRAIN DIFFERENCES IN FLUOXETINE-INDUCED SEXUAL DYSFUNCTION

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DEDICATION

To my beloved parents, Mr. Krishna Rao and Mrs. Mary Satyavathi, and my loving sister, Nirmala, and brother, Showri Raj, and my dear husband Mr. Johnson Joseph for their love, patience, support, encouragement and being my strength in my endeavors in life.

and

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ABSTRACT

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STRAIN DIFFERENCES IN FLUOXETINE-INDUCED SEXUAL DYSFUNCTION

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The present experiments were designed to examine strain differences in sexual dysfunction after acute fluoxetine treatment. Female Fischer and Sprague-Dawley rats were used. Three major experiments were performed. (1) Strain differences were examined in regularly cycling female rats and in ovariectomized rats hormonally primed with 0.067 µg/g estradiol benzoate and 3.333 µg/g progesterone following treatment with 5, 10, 15, 20 or 30 mg/kg fluoxetine. (2) The effects of the 5-HT_2 receptor antagonist, ketanserin 0.416, 0.5, 1, 2, 5 or 10 mg/kg, were compared in the two strains. (3) The combination of 10 mg/kg fluoxetine and l mg/kg ketanserin was examined. The major outcomes of this study were: (i) consistent with prior studies, fluoxetine reduced female rat sexual behavior in both hormonally-primed, ovariectomized and in naturally cycling rats; (ii) hormonally primed, ovariectomized rats were more sensitive to the lordosis inhibiting effects of fluoxetine than the intact, naturally cycling females; (iii) in both hormonally-primed and naturally cycling rats, Sprague-Dawley females were less sensitive to the lordosis-inhibiting effects of fluoxetine than Fischer females; (iv) a 5-

$HT_{2A/2C}$ receptor antagonist, ketanserin, reduced lordosis behavior in both strains with a slightly greater effect in Sprague-Dawley females, but the difference was modest in comparison to the strain differences in response to either fluoxetine, and (v) the combination of fluoxetine and ketanserin did not amplify negative effects on lordosis behavior relative to the individual drugs alone. From these experiments, it was concluded that Fischer female rats are more sensitive than Sprague-Dawley females to the lordosis inhibiting effects of fluoxetine and that the 5-HT_2 receptor, may not be involved. Potential mechanisms responsible for strain differences in the receptor activation are discussed.

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LIST OF ABBREVIATIONS

Word	Abbreviation
1. Analysis of Variance	ANOVA
2. Corticosterone	CORT
3. Corticotrophin Releasing Factor	CRF
4. Central Nervous System	CNS
5. Dorsal Raphe Nucleus	DRN
6. Estradiol Benzoate	EB
7. Hypothalamic-Pituitary-Adrenal	HPA
8. Paraventricular Nucleus	PVN
9. Progesterone	P
10. Serotonin	5-HT
11. Serotonin Transporter	SERT
12. Selective Serotonin Reuptake Inhibitor	SSRI

CHAPTER I

INTRODUCTION

Serotonin (5-HT) is a monoamine neurotransmitter that has been implicated in behaviors such as anxiety, depression and eating disorder. (Berridge et al., 2010; D'Souza and Craig, 2010; Lam et al., 2010). Depression is a common type of mental disorder affecting about 10% of the American population (Pratt and Brody, 2008), and women are more susceptible and twice more likely to show depression than men (Gregorian et al., 2002; Pratt and Brody, 2008). Although the etiology of depression may include dopamine and norepinephrine neurotransmission dysfunction (Dobson et al., 2003; Fuller and Snoddy, 1993), the major emphasis has been directed towards the 5-HT system.

Monoamine oxidase inhibitors and tricyclics were among the first pharmaceuticals that were prescribed for the treatment of depression (Carrasco and Van de Kar, 2003; Kehne et al., 1996). However, with the discovery of selective serotonin reuptake inhibitors (SSRIs), such as [±]-N-methyl-γ-[4-(trifluoromethyl)-phenoxy]benzenepropanamine (fluoxetine) (Prozac®), which showed less severe side effects and complications, SSRIs became the prescription of choice (Auger et al., 2001). The therapeutic effectiveness of SSRIs led to speculation about the importance of the serotonin transporter (SERT) in depression (Owens and Nemeroff, 1994). While SSRIs

are valuable therapeutic agents, they also produce sexual dysfunction in 30-80% of patients (Balon et al., 1993; Clayton, 2003; Michelson et al., 2000; Montejo-Gonzalez et al., 1997; Montgomery et al., 2002). Because there is a 2-3 week delay before patients begin to experience remission from symptoms of depression, patients may stop taking their medication to avoid these sexual complications (Balon et al., 1993; Rothschild, 2000).

Fluoxetine is one of the most widely prescribed of these antidepressants and is also among the drugs that produce the greatest amount of sexual dysfunction in human females (Clayton et al., 2006; Clayton, 2003). The sexual dysfunction is thought to result from the increase in extracellular 5-HT that results from SSRI blockage of SERT and studies in animal models have reinforced this conclusion.

In female rodents, sexual behavior consists of several aspects defined as attractivity, proceptivity and receptivity. Attractivity refers to the females' attractiveness to the male and includes visual or olfactory cues; proceptivity is the hopping, darting and ear wiggling displayed by females that draw attention of a male toward a female; and receptivity (or consummatory) behavior is the response elicited by the female's adoption of a posture that facilitates ejaculation by the male (Beach, 1976). The lordosis posture involves arching of the back, elevation of the neck and dorsoflexion of the tail. In female rats, lordosis is quantified as the lordosis quotient, calculated as the ratio of the total number of lordosis responses divided by the total number of mounts by the male times 100 (Beach, 1943) and is used to measure the degree of receptivity of the female. In animal models, compounds which increase 5-HT are known to decrease sexual behavior

(Miryala et al., 2012; Mos et al., 1999). Agents that increase extracellular 5-HT are inhibitory to lordosis whereas agents that decrease 5-HT are facilitatory (Mendelson and Gorzalka, 1985). In sub-primate females, sexual behavior is expressed during the proestrous stage of the estrous cycle (analogous to the menstrual cycle in human females) (Warner, 1927). Since increased extracellular 5-HT decreases lordosis behavior, it is not surprising that fluoxetine reduces sexual behavior. By blocking the SERT and resulting in an increase in extracellular 5-HT (Malagié et al., 1995; Tao et al., 2002), fluoxetine leads to increased activation of multiple 5-HT receptors, some of which may contribute to the sexual side effects. There are seven families of 5-HT receptors (5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7) and, within each family, there are several subtypes (Hoyer et al., 2002). Serotonergic activation of these receptor subtypes elicits different responses mediated via different signaling processes (Hoyer et al., 1987). Activation of 5-HT_{1A} receptors decreases female sexual behavior (Uphouse et al., 1994), while activation of 5-HT_2 receptors may increase the behavior (Maswood et al., 1996; Wolf et al., 1998).

Fluoxetine treatment also influences neuroendocrine responses by activation of 5-HT_{1A} and 5-HT_2 receptors, which play a major role in hypothalamic-pituitary-adrenal (HPA) function (Bagdy, 1995). One of the responses to the activation of the HPA includes an increase in corticotrophin releasing factor (CRF) from the parvocellular neurons of the hypothalamic paraventricular nucleus (PVN) (Jorgensen et al., 1998). Since CRF is known to inhibit lordosis behavior (Sirinathsinghji, 1986), it is possible that fluoxetine's HPA activation contributes to its effect on sexual behavior.

Regardless of the initial mechanism involved, fluoxetine is well associated with sexual dysfunction. However, all the characteristics of sexual dysfunction (for example, low sexual desire, anorgasm) are not observed in a single individual and not all individuals are affected (Clayton, 2002; Clayton, 2003; Strohmaier et al., 2011). Some individuals seem resistant to SSRI treatment while others show considerable clinical improvement (Rush et al., 2006). Similarly, in rodent animal models, there is evidence of strain differences in the effects of SSRIs (Scholl et al., 2010; Fernandez et al., 2003; Yalcin et al., 2008). Although mechanisms of these sexual side effects are unknown, they may involve the SSRI's effects on the serotonergic system so that individual differences in SSRI-induced sexual dysfunction could result from differences in functioning of the 5-HT system.

In spite of evidence for strain differences in the response to SSRIs and other 5-HT active compounds (Al Ahmed and Herbert, 2008; David et al., 2003; Sugimoto et al., 2008), less emphasis has been directed toward the evaluation of the sexual side effects so that a limited number of rat strains have been examined. Fischer females have a higher baseline extracellular 5-HT and are thought to be hyper responsive to stress while Sprague-Dawley females generally show a low responsivity to stress (Glowa et al., 1992; Sternberg et al., 1992). For example, Rosecrans et al. (Rosecrans et al., 1986) reported that 5-HT levels were increased more in Fischer than in Sprague-Dawley rats in response to foot shock and that Fischer rats had a higher plasma corticosterone (CORT) than Sprague-Dawley rats in response to the stressor (Rosecrans et al., 1986).

In a few studies, strains have been compared following treatment with 5-HT active drugs. Sprague-Dawley females are more sensitive than Fischer females to lordosis inhibition after treatment with a 5-HT_{1A} receptor agonist (Uphouse et al., 2002). However, the estrous cycle of Sprague-Dawley females was only moderately interrupted by daily treatment with fluoxetine, while the estrous cycle of Fischer females was robustly affected (Maswood et al., 2008; Sarkar et al., 2008). In addition, inhibition of lordosis behavior in Fischer females after fluoxetine was inhibited by prior treatment with the 5-HT_{1A} receptor antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide (WAY100635), suggesting that the lordosis inhibition after fluoxetine in Fischer females is mediated via the 5-HT_{1A} receptor. The apparently contrasting effects of fluoxetine and a 5-HT_{1A} receptor agonist in the two strains may challenge the assumption that the fluoxetine-induced decline of lordosis behavior is mediated via activation of 5-HT_{1A} receptors. Since, fluoxetine would lead to an increased activation of both 5-HT_{1A} and 5-HT_2 receptors, it is possible that the activation of 5-HT_2 receptors protects the Sprague-Dawley females against effects of lordosis inhibition mediated through the activation of 5-HT_{1A} receptors (Maswood et al., 1996). If 5-HT_2 receptors play a greater role in Sprague-Dawley than in Fischer females in the control of lordosis behavior, then, in Sprague-Dawley rats, 5-HT_2 receptors might compensate for the lordosis-inhibiting effects of 5-HT_{1A} receptor activation.

Alternatively, the apparent contradiction could result from the different models used for examination of the effects of fluoxetine and those of the 5-HT_{1A} receptor agonist. Estrous cycle differences in Fischer and Sprague-Dawley rats were examined in intact

females, while the effects of the 5-HT_{1A} receptor agonist, 8-hydroxy-2(di-n-propylamino-tetralin (8-OH-DPAT), were examined in ovariectomized, estradiol benzoate (EB) and progesterone (P) primed females. This difference may be important since both EB and P influence the number and/or function of 5-HT_1 and 5-HT_2 receptors (Sumner et al., 1999; Moses et al., 2000), uncouple 5-HT_{1A} receptors from G-proteins (Mize et al., 2003; Lu and Bethea, 2002; McQueen et al., 1997), and can influence the effects of 5-HT_{1A} and 5-HT_2 receptor agonists on lordosis behavior (Wilson and Hunter, 1985; Jackson and Uphouse, 1998; Sinclair-Worley and Uphouse, 2004). Therefore, it is important to examine the effects of fluoxetine in both intact and ovariectomized models in order to further identify the putative strain difference in the response to the SSRI.

In the following study, an attempt was made to understand the individual differences in response to fluoxetine by comparison of two rat strains - Fischer and Sprague-Dawley female rats - using the lordosis reflex as a model. The following hypotheses were tested:

1. Sprague-Dawley females are more sensitive than Fischer females to the lordosis inhibiting effects of acute fluoxetine. This hypothesis was not confirmed.
2. Lordosis inhibition is greater in Sprague-Dawley compared to Fischer females after blocking the 5-$HT_{2A/2C}$ receptors with ketanserin. This hypothesis was not confirmed.

3. Treatment with fluoxetine and ketanserin will amplify the lordosis inhibition in the two strains so that a strain difference will not exist. This hypothesis was not confirmed.

From these studies, we have found that Fischer female rats were more sensitive than Sprague-Dawley female rats to the lordosis-inhibiting effects of fluoxetine (Miryala et al., 2012). Although, Sprague-Dawley females were more sensitive to the 5-HT_{1A} receptor agonist, 8-OH-DPAT, blocking 5-HT_2 receptors with the 5-$HT_{2A/C}$ receptor antagonist, ketanserin, showed less strain differences. Finally, ketanserin and fluoxetine were combined and this effect was examined in the two strains. The combined treatment did not amplify the effects of ketanserin and strain differences were not present. Collectively, these studies are consistent with evidence that the 2 strains differ in 5-HT function and that 5-HT_{1A}, but not 5-HT_2 receptors, may differentiate the strains.

CHAPTER II

SPRAGUE-DAWLEY AND FISCHER FEMALE RATS DIFFER IN ACUTE EFFECTS OF FLUOXETINE ON SEXUAL BEHAVIOR

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ABSTRACT

Introduction. The selective serotonin reuptake inhibitor (SSRI), fluoxetine, leads to sexual dysfunction in a substantial proportion of women. In studies with the Fischer inbred rat, the 5-HT_{1A} receptor has been implicated in this sexual dysfunction. Whether this association with 5-HT_{1A} receptors holds for other rat strains is not known.

Aim. The effects of acute fluoxetine on sexual behavior in two strains of rats that differ in their response to a 5-HT_{1A} receptor agonist were examined. Whether the strain difference is comparable in naturally cycling and hormonally primed, ovariectomized rats was determined.

Main Outcome Measures. Lordosis to mount ratios, lordosis quality, and proceptive behaviors were quantified. Sprague-Dawley and Fischer females were compared on each of these measures. The IC_{50} for inhibition of lordosis behavior was determined.

Methods. Proestrous rats and ovariectomized rats, hormonally primed with estradiol benzoate and progesterone, were treated with varying doses of fluoxetine. Sexual

behavior was examined before and after treatment with the SSRI.

Results. In both the intact and the hormonally-primed, ovariectomized model, Sprague-Dawley females were less sensitive to the effects of fluoxetine on sexual behavior. In both groups, fluoxetine showed dose-dependency in behavioral inhibition, but a higher dose was required for Sprague-Dawley than for Fischer females. Naturally cycling, proestrous rats required a higher dose of fluoxetine than hormonally-primed ovariectomized rats to produce significant inhibition of sexual behavior. Thus, the strain difference in the response to fluoxetine does not parallel strain differences in the response to a 5-HT_{1A} receptor agonist.

Conclusions. Acute treatment with fluoxetine inhibits lordosis behavior in both Fischer and Sprague-Dawley females and the strain difference cannot be explained by reported strain differences in the response to a 5-HT_{1A} receptor agonist. Fluoxetine's inhibition of female rat sexual behavior may involve effects of the SSRI in addition to activation of the 5-HT_{1A} receptor.

Key Words: Rat strains, female sexual behavior, SSRI, estrogen, progesterone, proestrous

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Introduction

After its original marketing in the 1980s, Prozac® (fluoxetine) became one of the most frequently prescribed medications for the treatment of depression and other disorders of special significance to women [1, 2]. Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) thought to exert its therapeutic effect, in part, through blocking of the serotonin transporter (SERT) and consequent reduction of serotonin reuptake into the nerve terminal [3, 4]. However, in spite of the clinical effectiveness of SSRIs for the treatment of mood disorders, the 2 to 3 week delay between onset of treatment and clinical effectiveness, coupled with the emergence of sexual side effects, can lead patients to discontinue treatment prior to relief from their original clinical symptoms [5-7].

Approximately 30-50% of women taking antidepressants experience some kind of sexual dysfunction [5] and there is a higher probability of such sexual side effects with SSRI treatments leading to suggestions that the sexual dysfunction may involve the drugs' effects on the serotonergic system [5, 8, 9]. However, compared to research on male sexual dysfunction, investigation of the mechanisms responsible for SSRI-induced female sexual dysfunction has been limited and it is not clear why some females experience such sexual side effects while others do not. From animal models, it is well established that manipulations, including fluoxetine, that elevate CNS serotonin have inhibitory effects on female rat sexual behavior [10, 11] and activation of the serotonin 1A (5-HT_{1A}) receptor has been implicated in 5-HT-mediated sexual inhibition [11]. Activation of the 5-HT_{1A} receptor also contributes to the acute effect of fluoxetine on

female rat sexual behavior [12]. However, this association may not hold for the SSRI, paroxetine [13], and whether or not this relationship holds across rat strains has not been determined. In fact, the possibility of rat strain differences in fluoxetine's effect on sexual behavior has received little investigation.

There is, though, evidence that rat and mouse strains vary in their response to several effects of fluoxetine [14-17] and fluoxetine-induced disruption of the female rat's estrous cycle is more prevalent in the Fischer inbred strain than the Sprague-Dawley strain [18, 19]. In contrast, Sprague-Dawley females show greater sensitivity than Fischer females to the disruptive effects of a 5-HT_{1A} receptor agonist on sexual behavior [20]. This is surprising since the Sprague-Dawley strain that is more sensitive to the 5-HT_{1A} receptor agonist would have been expected to exhibit the greater vulnerability to sexual side effects of fluoxetine. However, it is important to note that effects of a 5-HT_{1A} receptor agonist were investigated in hormonally-primed ovariectomized rats while strain differences in the effects of fluoxetine on estrous cyclicity were investigated in naturally cycling female rats. Therefore, it is possible that the direction of the strain difference in the female's response to fluoxetine is not the same in the naturally cycling and hormonally-primed, ovariectomized model. The following experiments were designed to investigate this possibility. Portions of these studies were previously reported at the Annual Meeting of the Society for Neuroscience [21].

Materials and Methods

Materials

Estradiol benzoate (EB), progesterone (P), sesame seed oil, and the selective serotonin reuptake inhibitor, methyl [3-phenyl-3-4-(trifluoromethyl)-phenoxy] propyl] ammonium chloride (fluoxetine), were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Isoflurane (AErrane®) and suture materials were purchased from Butler Schein Animal Health (Dublin, OH). Food (Rodent Lab Diet 5001) was purchased from Lab Animal Supply (Highland Village, Texas). All other supplies were purchased from Fischer Scientific (Houston, TX).

General Methods

All procedures were in accordance with the NIH Guide for the Care and Use of Animals in Research and were approved by the Institutional Animal Care and Use Committee at Texas Woman's University.

Animals and Housing Conditions

Female Fischer and Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA) and housed 2 per cage in standard shoebox caging (45.72 × 24.13 × 2.59 cm). The housing area was maintained at 25 °C and 55% humidity with lights on from 12:00 midnight to 12:00 noon. Food and water were available *ad lib*. Age of the animals varied within experiments but was always matched between Fischer and Sprague-Dawley rats and was counterbalanced across treatment conditions.

Behavioral Testing Procedures

On the day of testing, rats were pretested for sexual behavior during the dark portion of the light/dark cycle (between 2 and 4 pm) as previously described [22]. Females were sexually naïve prior to testing and were used only a single time. Rats were placed into the home cage of a sexually active Sprague-Dawley male and behavior was monitored until 10 mounts had occurred. Females were then injected with fluoxetine and tested 30 min later for Experiments 1, 2, and 4 or after 5 min for Experiment 3. Sexual receptivity was measured as the lordosis/mount (L/M) ratio (number of lordosis reflexes by the female divided by the number of mounts by the male). Lordosis quality was measured on a scale of 1 to 4 as previously described [22] and was computed as the sum of individual lordosis quality scores divided by the number of lordosis responses. Rats with only minor arching of the back received a score of 1.0; a standard arch with elevation of the head was scored as 3.0; an intermediate arch without head elevation was scored as 2.0; and an exaggerated arch with elevation of the front legs was scored as 4.0. If the female failed to lordose, no quality score was given. The number of mounts by the male was recorded to assure that females received comparable stimuli. Proceptivity was measured by the presence of hopping and darting behavior. For L/M ratios, lordosis quality, and mounts, data were recorded for the pretest and consecutive intervals after fluoxetine. For proceptivity, the presence or absence of the behavior was recorded and females were categorized as either proceptive or not proceptive.

Statistical Procedures

For Experiments 1 and 2, L/M ratios, lordosis quality scores and number of mounts were computed for the pretest and the 15 min test period after fluoxetine. Data were evaluated by repeated measures ANOVA with strain and dose as independent factors and before or after fluoxetine as the repeated factor. Data for experiments 3 and 4 were divided into consecutive 5 min (Experiment 4) or 10 min (Experiment 3) intervals and were evaluated by repeated measures ANOVA with time relative to the fluoxetine injection as the repeated factor and strain as the independent factor. Proceptivity data were compared by Chi-Square or Fisher's Exact Test procedures. In the first 2 experiments, L/M data were subjected to regression on dose for determination of the relationship between dose and L/M ratios and for estimation of the IC_{50}. IC_{50}, the dose that leads to 50% inhibition of the maximum effect [23], was defined as an L/M ratio of 0.5. SPSS versions 15.0 (for PC) or 17.0 (for Macintosh) were used for data analysis. Post-hoc Tukey test comparisons were performed manually and were limited a priori to (a) comparisons of data before and after fluoxetine (within treatment) and (b) comparison of strains (within dose and test interval) [24]. An alpha level of 0.05 was required for rejection of the null hypothesis.

Specific Methods

Experiment 1: Intact, Proestrous Rats

Fischer and Sprague-Dawley rats were age-matched for the experiment and were 17-20 weeks of age at the time of testing. Approximately 2 weeks after their arrival at TWU, vaginal cyclicity was monitored daily as previously described [22]. Females

showing at least two consecutive 4 to 5 day estrous cycles were tested on the afternoon of proestrous. Proestrus was defined as a relative scarcity of leukocytes and a high density of nucleated and cornified cells. Animals were selected on each test day on the basis of their prior vaginal smear history and their vaginal smear on the day of testing with the additional requirement that Fischer and Sprague-Dawley females be represented on every testing day.

On the day of testing, the means ± S.E. body weights of intact Fischer and Sprague-Dawley rats were 160.1 ± 1.8 and 264.9 ± 4.6 g, respectively. On the afternoon of proestrous, females were pretested for sexual behavior and immediately injected intraperitoneally (ip) with 10, 15, 20 or 30 mg/kg of fluoxetine hydrochloride. Thirty min later, females were again tested in the home cage of a sexually active male. Testing continued for 15 consecutive min. Data were compared for the pretest (before fluoxetine) and a 15 min test period 30 through 45 min after fluoxetine.

Experiment 2: Hormonally Primed, Ovariectomized Rats

Approximately two weeks after their arrival, females were ovariectomized under AErrane® anesthesia as previously described [22]. Ten to 14 days later, rats were hormonally primed with estradiol benzoate (EB) followed 48 hr later with progesterone (P). Because Fischer and Sprague-Dawley females differ in body weight, hormones were administered by body weight (0.067 µg /g EB and 3.333 µg/g P) and were the same doses as previously used in the strain comparison with a 5-HT_{1A} receptor agonist [20]. These doses are roughly equivalent to 10 µg EB and 500 µg P for a 150 g Fischer female. EB and P were dissolved in sesame seed oil and administered subcutaneously (sc) in a

volume of 0.1 ml/150 g body weight. On the day of testing, Fischer and Sprague-Dawley females were 15-19 weeks of age and weighed 168.8 ± 1.4 and 276.2 ± 3.4 g, respectively.

Immediately following the pretest, females were injected with 5, 10, 15 or 20 mg/kg fluoxetine. A lower dose range was chosen for ovariectomized females because of an expectation that the ovariectomized rats would be more sensitive to the effects of fluoxetine. This expectation was derived from prior work demonstrating that sexual behavior of naturally cycling females is more resistant to disruption by both chemical and experimental factors than is that of hormonally-primed ovariectomized rats [11, 20, 25]. Although exogenous hormonal treatment is effective in priming for sexual behavior, it does not replicate the endogenous pattern of hormonal secretion that is probably optimal for maintenance of reproductive activity. Thirty min after fluoxetine, rats were retested for sexual behavior as in Experiment 1. Data were compared before and for 15 consecutive min of testing 30 through 45 min after fluoxetine.

Experiment 3: Rapid Effects of Fluoxetine in Hormonally Primed, Ovariectomized Rats

Females were ovariectomized and hormonally primed as for Experiment 2. Rats fell into two different age groups (14-15 weeks or 20 weeks) and all treatment conditions were represented in both age groups. Fischer and Sprague-Dawley females weighed 160.2 ± 3.07 and 257.2 ± 7.94, respectively, on the day of testing. After the pretest for sexual behavior, females were injected with 15 mg/kg fluoxetine. Five min later, rats were tested for sexual behavior for 40 consecutive min (to allow overlap with rats tested 30 min after fluoxetine). Data were computed for the pretest and for the 4 continuous 10

intervals after initiation of testing. Sexual behavior and motor disturbances were monitored. Females were judged to exhibit motor disturbance when they showed immobility, disordered gait, or difficulty with ambulation. L/M ratios and lordosis quality were compared by repeated measures ANOVA with test interval as the repeated factor. Motor disturbance was evaluated by Chi-Square procedures.

Experiment 4: Effect of Hormonal Priming per Rat

In Experiments 2 and 3, hormonal priming was based on body weight so that the larger Sprague-Dawley females received higher absolute amounts of hormones. Both estradiol and progesterone can influence the serotonergic system [26] and progesterone can reduce effects of fluoxetine on lordosis behavior [12]. Therefore, in the final study, rats were treated as described for Experiment 2 but doses of 10 μg EB and 500 μg P per rat were given to both strains. EB and P were dissolved in sesame seed oil and administered subcutaneously (sc) in a volume of 0.1 ml/rat. At the time of testing, rats were 18-20 weeks of age and weighed 166.8 ± 2.23 and 280.1 ± 5.47, respectively, for Fischer and Sprague-Dawley females. After the pretest for sexual behavior, rats were injected with 15 mg/kg fluoxetine. Behavioral testing was initiated 30 min after progesterone and continued for 15 consecutive min. Data were computed for the pretest and 3 continuous 5-min intervals after fluoxetine and were analyzed by repeated measures ANOVA as for Experiment 3.

Outcome Measures

Lordosis to mount ratios, lordosis quality, and proceptive behaviors were quantified. Hormonally-primed and naturally-cycling Sprague-Dawley and Fischer females were

compared on each of these measures. The IC_{50} for inhibition of lordosis behavior was determined.

Results

Experiment 1: Intact, Proestrous Females

In intact, proestrous females, there was a significant main effect of strain ($F_{1, 40}$ = 10.71, $p \leq 0.002$) and dose on L/M ratios after fluoxetine (Figure 2.1; $F_{3, 40} = 6.77$, $p \leq 0.001$). Strains did not differ in L/M ratios before fluoxetine. L/M ratios declined after fluoxetine leading to a significant effect of time (e.g. before or after fluoxetine) that was due primarily to effects in Fischer females ($F_{1, 40} = 45.68$, $p \leq 0.001$). There was a significant time by strain interaction ($F_{1,40} = 11.55$, $p \leq 0.002$) as well as a significant time by dose interaction ($F_{3,40} = 7.23$, $p \leq 0.001$). L/M ratios of Fischer rats were significantly different from their pretest after 15, 20 and 30 mg/kg fluoxetine ($q_{40, 4}$, respectively, = 4.03, 6.59 and 8.99, $p \leq 0.05$). In contrast, L/M ratios of Sprague-Dawley females differed from their pretest only after 30 mg/kg ($q_{40, 4} = 4.64$, $p \leq 0.05$). L/M ratios of Fischer rats were significantly lower than those of Sprague-Dawley rats at the 20 and 30 mg/kg doses (respectively, $q_{40, 4} = 5.50$ and 4.35, $p \leq 0.05$).

There was a significant linear relationship between dose and L/M ratios for both strains (for Fischer and Sprague-Dawley, respectively, $r = 0.78$ and 0.52, $p \leq 0.001$). The IC_{50} for Fischer and Sprague-Dawley females, respectively, was 26.0 and 64.4 mg/kg.

Females that failed to show lordosis behavior were omitted from the analysis for lordosis quality. For the remaining rats, fluoxetine had relatively minor effects (data not

shown). There was a significant effect of dose ($F_{3,38} = 3.43$, $p \leq 0.026$) and an interaction between strain and dose ($F_{3,38} = 7.14$, $p \leq 0.001$) due to a lower lordosis quality in Fischer females after 15 mg/kg fluoxetine and in Sprague-Dawley females after 30 mg/kg; but even after 30 mg/kg, Fischer and Sprague-Dawley rats, respectively, showed relatively high lordosis quality (average scores of 2.79 ± 0.09 and 2.27 ± 0.20).

Prior to injection with fluoxetine, 84% of Fischer and 100% of Sprague-Dawley females showed proceptive (hopping and darting) behavior. In both strains, fluoxetine dose-dependently reduced proceptivity (Chi Square values for Fischer and Sprague-Dawley, respectively, were 28.72 and 40.62, df = 4, $p \leq 0.001$; see Figure 2.2). However, Fischer females were affected at a lower dose than Sprague-Dawley females. Fischer females were significantly different from their pretest after 20 and 30 mg/kg fluoxetine while Sprague-Dawley females differed only after 30 mg/kg. As a consequence, the strains differed significantly only at 20 mg/kg fluoxetine (Chi Square = 7.63, df = 1, $p \leq 0.006$).

The strain difference in lordosis behavior was not due to differential mounting by the males ($p > 0.05$). The mean ± S.E. number of mounts during the 15 min after injection for Fischer and Sprague-Dawley rats, respectively, for 10, 15, 20 and 30 mg/kg fluoxetine, were: 14.83 ± 2.52, 19.28 ± 3.03, 24.3 ± 3.91, 18.5 ± 3.62 and 15.16 ± 1.51, 17.16 ± 2.27, 21.0 ± 2.9, 17.66 ± 4.57.

Experiment 2: Hormonally Primed, Ovariectomized Females

Strain differences in hormonally-primed, ovariectomized rats were similar to those of intact females with Fischer females showing the greater decline in L/M ratios

after fluoxetine (ANOVA for strain, $F_{1, 54} = 5.27$, $p \leq 0.026$; see Figure 2.3). There was also a significant main effect of dose ($F_{3, 54} = 9.39$, $p \leq 0.001$) and a strain by dose interaction ($F_{3, 54} = 3.08$, $p \leq 0.035$). The time-dependent decline (before versus after fluoxetine) in L/M ratios ($F_{3, 54} = 94.59$, $p \leq 0.001$) interacted significantly with both strain ($F_{1, 54} = 14.65$, $p \leq 0.001$) and dose ($F_{3, 5} = 14.11$, $p \leq 0.001$), and there was a significant 3-way interaction ($F_{3, 54} = 2.82$, $p \leq 0.048$). L/M ratios of Fischer females were significantly different from their pretest after 10, 15 and 20 mg/kg fluoxetine (all $q_{54,4} \geq 4.97$, $p \leq 0.05$) while L/M ratios of Sprague-Dawley females differed from their pretest only after 20 mg/kg ($q_{54,4} = 5.39$, $p \leq 0.05$). L/M ratios of the two strains differed significantly after 15 and 20 mg/kg (respectively, $q_{54,4} = 4.15$ and 8.16, $p \leq 0.05$).

As for intact females, there were significant linear regressions between dose and L/M ratios (respectively, for Fischer and Sprague-Dawley rats, $r = 0.82$ and 0.56, $p \leq 0.001$). The IC_{50} was 16.93 and 39.5 mg/kg for Fischer and Sprague-Dawley females, respectively.

Females that did not show lordosis after fluoxetine were omitted from the data analysis. For the remaining rats, lordosis quality decreased after fluoxetine (ANOVA for pre versus post, $F_{1, 54} = 49.58$, $p \leq 0.001$; data not shown) and there was a significant interaction between time and dose of fluoxetine ($F_{3, 54} = 4.57$, $p \leq 0.006$). No other effects were significant. Lordosis quality after 20 mg/kg fluoxetine ranged from a high of 2.4 ± 0.198 to a low of 2.1 ± 0.75 in Fischer and Sprague-Dawley rats, respectively.

Before injection with fluoxetine, 65.62% of Fischer females and 80% of Sprague-Dawley females showed proceptive behavior which declined after fluoxetine (Chi Square

= 9.44 and 12.65, respectively, for Fischer and Sprague-Dawley females, df = 4, $p \leq 0.05$; see Figure 2.4). Proceptivity of Fischer females was lower than their pretest at every dose of fluoxetine but, because of relatively low proceptivity in the pretest, showed a significant difference only after 15 and 20 mg/kg of fluoxetine (Fisher's Exact Test, $p \leq 0.03$). Proceptivity of Sprague-Dawley females declined at 10, 15 and 20 mg/kg fluoxetine but were only significantly different from their pretest at 10 mg/kg (Fischer's Exact Test, $p \leq 0.014$). Since proceptivity declined in both strains, only after 5 mg/kg was there a significant strain difference in proceptivity (Chi Square = 5.33, df = 1, $p \leq 0.02$) and this primarily reflected the 100% incidence of proceptivity in Sprague-Dawley females at this dose.

Strain differences in lordosis behavior did not result from differences in number of mounts from the male. The mean ± S.E. number of mounts during the 15 min after injection for Fischer and Sprague-Dawley rats, respectively, for 5, 10, 15 and 20 mg/kg fluoxetine, were 27.25 ± 2.84, 16.62 ± 1.51, 20.44 ± 1.56, 27.25 ± 4.33 and 19.37 ± 2.18, 25.57 ± 4.11, 23.28 ± 2.66, 18.75 ± 2.45. None of the main effects were significant but there was a significant strain by dose interaction ($F_{3, 54} = 3.58 \leq 0.020$) due to slightly fewer mounts of Sprague-Dawley females at 5 mg/kg and slightly more mounts at 10 mg/kg.

Ovariectomized rats appeared to be more sensitive than intact rats to the lordosis-inhibiting effects of fluoxetine. Therefore, a post facto comparison of the two data sets was performed on the L/M ratios after injection with 10, 15 or 20 mg/kg fluoxetine (the doses present in both Experiments 1 and 2). There was the expected main effect of strain

($F_{1,70}$ = 19.98, $\leq$ 0.001), dose ($F_{2,70}$ = 8.32, p $\leq$ 0.001) and their interaction ($F_{2,70}$= 5.03, p $\leq$ 0.009). As anticipated from the dose response analyses for Experiments 1 and 2, type of animal (e.g. ovariectomized or intact) was also a significant factor ($F_{1,70}$ = 14.86, p $\leq$0.001)

Experiment 3: Rapid Effects of Fluoxetine in Hormonally Primed, Ovariectomized Rats

The next experiment was designed to determine if strain differences examined 30 min after fluoxetine resulted from different rates of recovery from the immediate effects of fluoxetine on motor function. Continuous behavioral monitoring began 5 min after injection with 15 mg/kg fluoxetine. As expected, females showed motor disturbances, characterized primarily by immobility, early after treatment (see Table 2.1). Surprisingly, a greater proportion of Sprague-Dawley females showed evidence of motor disturbance than did Fischer females; but this difference was not significant (Chi Square, p > 0.05). Probably because of this immobility (especially 10 to 20 min after injection), males expressed low interest in the females so that mounting did not occur for every female in every 5-min interval. In particular, 4 Sprague-Dawley and 1 Fischer female had no mounts during at least one 5-min test interval after treatment with fluoxetine so the data were collapsed into 10-min intervals for analysis.

The rapid effects of fluoxetine on L/M ratios are shown in Figure 2.5. Compared to Experiment 2 when testing was delayed for 30 min after injection (Figure 2.3), Sprague-Dawley females had lower L/M ratios when testing began 5 min after fluoxetine. Both strains, therefore, showed robust declines in L/M ratios within the first 15-20 min after fluoxetine, and the main effect of strain was not significant ($F_{1, 17}$ = 0.44, p $\leq$ 0.05).

There was a significant effect of time ($F_{4, 68} = 24.44$, $p \leq 0.001$) but not a significant interaction between time and strain ($F_{4, 68} = 0.48$, $p \leq 0.05$). In contrast to behavior tested 30 min after fluoxetine, with the immediate initiation of testing, L/M ratios of both strains were significantly different from the pretest at every test interval (all $q_{5.68} \geq 3.97$, $p \leq 0.05$). For both strains, L/M ratios gradually increased after the early nadir to reach L/M ratios at the end of testing that were similar in Fischer females to those seen in Experiment 2 but lower in Sprague-Dawley females.

For lordosis quality, there were several missing intervals due to L/M ratios of zero so data were evaluated by repeated measures ANOVA with lordosis quality before and after fluoxetine as the repeated factors. There was a significant decline in lordosis quality after injection (ANOVA for time $F_{1, 21} = 84.76$, $p \leq 0.001$) but neither the strain ($F_{1, 21} = 0.46$, $p > 0.05$) nor the strain by time interaction ($F_{1, 21} = 1.25$, $p > 0.05$) were significant. The means ± S.E. lordosis quality for Fischer females before and after fluoxetine, respectively, were 2.9 ± 0.05 and 1.69 ± 0.17; for Sprague-Dawley, lordosis quality before and after fluoxetine were 2.7 ± 0.05 and 1.7 ± 0.18, respectively.

Proceptivity was reduced by the SSRI and Fischer rats were more severely affected and for a longer time interval than Sprague-Dawley females (Table 2.1) [strain differences were present at the 20-25 min and 40-45 min intervals (Fischer's Exact test, $p < 0.05$)]. When the relationship between immobility and proceptivity was examined, the two variables were inversely correlated in Sprague-Dawley females at the 10 to 15, 20 to 25, and 40 to 45 min intervals (respectively, $r = -0.74$, -0.69, and -0.67; $p \leq 0.05$). For

Fischer females, a significant correlation was present only at the 10 to 15 min interval ($r = -0.47$, $p \leq 0.05$).

Experiment 4: Effects of Hormonal Priming per Rat

In a final experiment, ovariectomized Fischer and Sprague-Dawley females were primed with 10 µg EB and 500 µg P per rat to determine if strain differences resulted from the greater amount of hormones received by Sprague-Dawley females when hormones had been administered relative to body weight. As shown in Figure 2.6, strain differences were still evident with Sprague-Dawley females being less sensitive to fluoxetine than Fischer females. However, in contrast to Experiment 2, the strain difference was present only early during testing.

There was a significant effect of time ($F_{3,60} = 11.48$, $p \leq 0.001$) and a significant strain by time interaction ($F_{3,60} = 4.76$, $p \leq 0.005$) for L/M ratios. Ratios for Fischer females were significantly different from their pretest during the 30-35 and 35-40 min intervals after fluoxetine ($q_{60,8} \geq 4.36$, $p \leq 0.05$) while ratios for Sprague-Dawley females were never significantly different from their pretest. The apparent differences between Experiment 2 and Experiment 4 resulted from a slightly lower L/M ratio of Sprague-Dawley females in Experiment 4 and the unusually high L/M ratio of Fischer females during the last test interval.

For lordosis quality, 1 Sprague-Dawley and 4 Fischer females had L/M of zero during at least one test interval. For remaining rats, there was a decrease in lordosis quality (data not shown) after fluoxetine ($F_{3,45} = 2.87$, $p \leq 0.046$) but no other effects were significant. Similarly, the only significant effect for mounts was for time ($F_{3,66} =$

7.96, $p \leq 0.001$). The average numbers of mounts per 5 min test interval after fluoxetine were 5.44 ± 0.96 and 7.5 ± 0.96 for Fischer and Sprague-Dawley females, respectively.

Discussion

Three major observations were made in these studies: (1) both lordosis behavior and proceptivity were reduced by fluoxetine; (2) hormonally-primed, ovariectomized females were more sensitive to the antidepressant than naturally cycling, intact females; and (3) both hormonally-primed, ovariectomized and intact Sprague-Dawley females were less sensitive to the disruptive effects of fluoxetine on sexual behavior than were Fischer females. These studies were originally initiated because Sprague-Dawley females show greater sensitivity to the lordosis-inhibiting effects of a 5-HT_{1A} receptor agonist [20] and 5-HT_{1A} receptors have been implicated in fluoxetine's antidepressant actions [27, 28] and in the SSRI's reduction of female rat sexual behavior [12]. It was, therefore, expected that Sprague-Dawley females might also be more sensitive to the effects of fluoxetine on sexual behavior. This clearly was not the case and challenges the role of 5-HT_{1A} receptors in fluoxetine's effect on sexual behavior.

However, relative to Sprague-Dawley or some other rat strains, Fischer rats are reported to have more SERT mRNA in the dorsal raphe nucleus [29]. If this translates into greater SERT activity in dorsal raphe of Fischer females, then fluoxetine would be expected to (a) increase extracellular 5-HT to a lesser extent in the vicinity of the dorsal raphe and (b) lead to a lesser activation of somatodendritic 5-HT_{1A} autoreceptors that function to reduce firing of 5-HT neurons. Consequently, extracellular 5-HT in brain areas such as the mediobasal hypothalamus that are important in the control of female

sexual behavior could be elevated more by fluoxetine in Fischer than in Sprague-Dawley females. In addition, because of a putative hyper functional 5-HT system in Fischer rats [29, 30], a lower dose of fluoxetine might be expected to reduce lordosis behavior. Such differences in SERT activity might account for the reversal of strain difference profiles found here after fluoxetine compared to that previously reported for a 5-HT_{1A} receptor agonist.

This possibility is even more interesting since Sprague-Dawley females appeared to have greater motor disturbance than Fischer females. Serotonin's effect on several indices of motor function is thought to result from effects on the descending 5-HT system [31, 32] while sexual behavioral effects may result from effects on ascending 5-HT systems [11]. A potential dissociation between fluoxetine effects on sexual behavior and motor function was seen in the current study where Sprague-Dawley females showed more motor effects than Fischer females.

The hyper functional HPA axis of Fischer rats could also contribute to the strain difference in the response to fluoxetine. Relative to several other rat strains, Fischer rats are thought to be more HPA responsive [30, 33], have a higher [34] and longer duration [35] corticosterone response to stress, and are regarded to be a highly emotionally reactive strain [36]. Acute treatment with fluoxetine is associated with increased activation of the hypothalamic-pituitary-adrenal axis [37] and adrenal corticosterone has been implicated in some effects of fluoxetine [15]. Therefore, fluoxetine's acute anxiogenic action may be more disruptive for Fischer than for Sprague-Dawley females.

The observation that ovariectomized females were more sensitive than intact females to the effects of fluoxetine is not surprising since the endogenous sequence of hormonal priming is probably optimal for both facilitation of sexual behavior and for resistance to its disruption. Relative to other stages of the estrous cycle, proestrous females show a blunted response to SSRIs as measured either by micro dialysis of the mediobasal hypothalamus [38] or by in vivo chronoamperometry of the hippocampus [39]. In the naturally cycling female, both estrogen and progesterone likely contribute to the smaller response to SSRIs by their modulation of SERT activity and/or by modulation of serotonin receptor number and/or function [26]. Although such hormonal modulation also occurs in ovariectomized, hormonally-primed females, the temporal characteristics of the priming mimic but do not replicate that of the naturally cycling animal.

In ovariectomized rats, both EB and P alter the behavioral and neurochemical responses to fluoxetine [40-43]. In EB-primed ovariectomized Fischer rats, P was reported to attenuate the lordosis-inhibiting effects of fluoxetine by shifting fluoxetine's dose response curve to the right [12] and fluoxetine's reduction of sexual behavior can be reduced by the progesterone metabolite, allopregnanolone [44]. Therefore, potential strain differences in gonadal steroids could influence the strain-dependent response to fluoxetine. It is important to note that Fischer and Sprague-Dawley females were age-matched for these experiments so that Sprague-Dawley females weighed approximately 1.5 times that of Fischer females. In Experiments 2 and 3, with ovariectomized rats, both EB and P were administered per body weight so that Sprague-Dawley females received greater absolute amounts of the hormones than their Fischer counterparts. It is possible

that the greater amount of hormones contributed to the lower effect of fluoxetine in Sprague-Dawley rats. Indirect evidence for hormonal modulation of SERT activity has been reported for both Fischer [38] and Sprague-Dawley [39] females and effects of gonadal hormones on serotonin receptor function have been reported for both strains [45, 46]. In the current experiment, when both strains received the same dose of hormones (10 µg/rat EB and 500 µg/rat P), the strain difference was present but was not persistent throughout the 15 min testing. The absence of a strain difference in the latter parts of the 15 min testing was a reflection of lower L/M ratios in Sprague-Dawley females given the lower concentrations of hormonal priming while L/M ratios of Fischer females were slightly higher than in prior experiments. Although this outcome could evidence a slight protective effect of the higher hormonal priming in Sprague-Dawley females, the fact that both the naturally-cycling and hormonally-primed, ovariectomized Fischer rats showed heightened sensitivity to fluoxetine makes it unlikely that differential responses to exogenous hormonal priming is the only explanation for the strain difference. In the few studies in which endogenous levels of hormones have been compared in Fischer and Sprague-Dawley females, there has been little evidence of consistent strain differences in plasma levels of both estradiol and progesterone [47, 48]. Nevertheless, such consideration deserves further examination since there is some evidence that chronic treatment with SSRIs may reduce serum levels of gonadal hormones [49, 50].

Estradiol is essential for facilitation of lordosis behavior while progesterone is not required but is more important for the occurrence of proceptive behavior [51]. The hormonal priming used in the present study is sufficient to elicit high levels of lordosis

responding in Fischer females but is not adequate for facilitation of proceptivity [22]. The fact that both lordosis behavior and proceptivity were reduced by fluoxetine challenges a simple hormonal explanation for the strain difference seen in the current study. Nevertheless, we cannot rule out the possibility that the strains differ in the degree to which the gonadal hormones modulate their serotonergic and/or other neuronal system.

Regardless of the ultimate explanation for the strain differences, the current findings illustrate the potential importance of genetic differences in the vulnerability for development of SSRI-induced sexual dysfunction. It is increasingly recognized that genetic differences contribute to both the vulnerability for development of mood disorders and for the therapeutic efficacy of SSRIs [52-54]. Similarly, differences in the behavioral and neurochemical responses to SSRIs have been reported for a variety of mouse and rat strains [55, 56]. Less attention has been focused on the possibility of genetic contributions for vulnerability to SSRI-induced sexual side effects. Although some 30-50% of females that are treated with SSRIs may develop some form of sexual dysfunction, the remaining 50-70% does not. The factors responsible for resistance to these sexual side effects are not known. However, Bishop and colleagues have described evidence that genetic polymorphisms in the promoter regions of the 5-HT_{2A} receptor gene or in the SERT gene may influence vulnerability to SSRI-associated sexual dysfunction [57, 58] but not all reports agree [59]. Other investigators have implicated individual differences in the effect of SSRIs on the P450 2D6 isoenzyme leading to accumulation of higher concentrations of the drugs [60]. Clearly, the ideal would be to differentiate those factors that are required for the therapeutic efficacy of SSRIs from those which lead to

sexual side effects. The differential vulnerability of Fischer and Sprague-Dawley females to SSRI-induced inhibition of female sexual behavior could, therefore, be valuable in identifying these factors.

Conclusions

Fischer inbred female rats are more sensitive than Sprague-Dawley females to the acute sexual side effects of fluoxetine. This difference appears to be independent of gonadal hormones.

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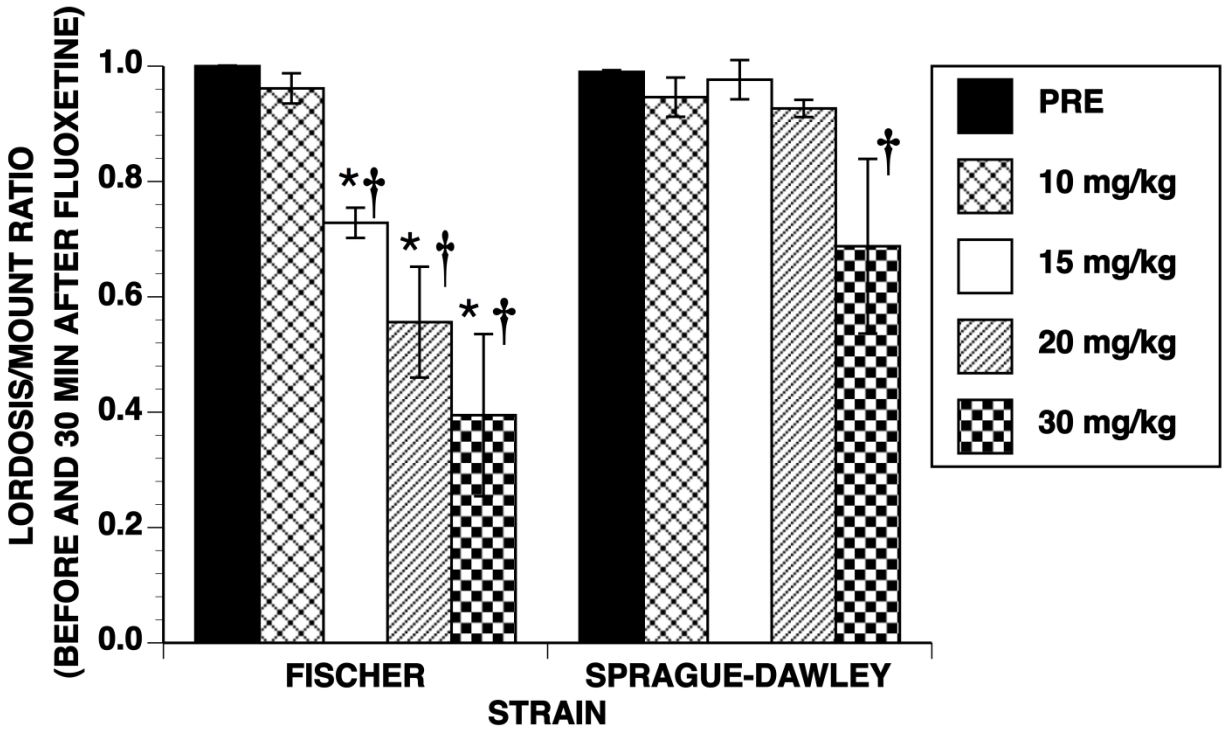

Figure 2.1 Fluoxetine dose-dependently decreased L/M ratios in intact rats. Proestrous Fischer (F) and Sprague-Dawley (S-D) females were pretested for sexual behavior and then injected ip with 10, 15, 20 or 30 mg/kg fluoxetine. Thirty min later, sexual behavior was again monitored for 15 consecutive min. Data are the mean ± S.E. lordosis/mount (L/M) ratios before (PRE) and for the 15 min after fluoxetine. Ns for Fischer rats at 10, 15, 20 and 30 mg/kg fluoxetine, respectively, were 6, 7, 6 and 6; Ns, respectively, for Sprague-Dawley rats were 6, 6, 5, and 6. * indicates significant difference between Fischer and Sprague-Dawley rats within dose of fluoxetine; † indicates significant difference from the pretest, within strain.

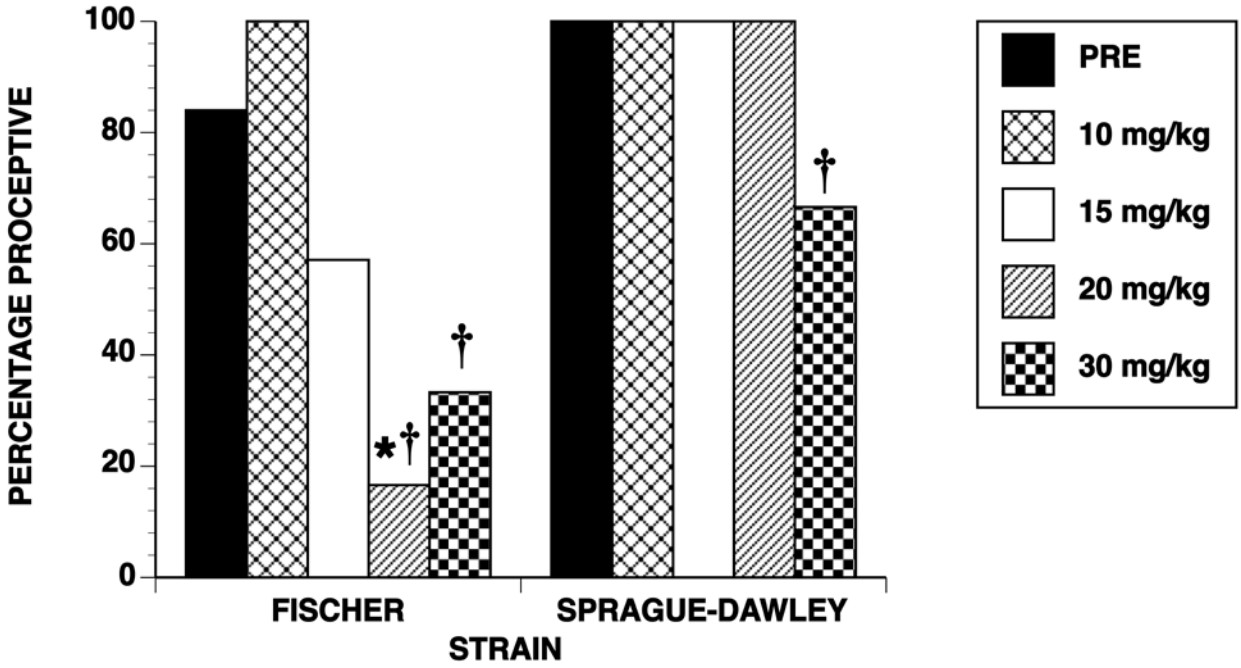

Figure 2.2 Dose-dependent effects of fluoxetine on proceptivity of intact rats.

Data are for the same rats shown in Figures 2.1 and are the percentage of rats that showed evidence of proceptivity before (PRE) and after fluoxetine treatment. * indicates significant strain differences within dose of fluoxetine. † indicates significant difference from the pretest, within strain.

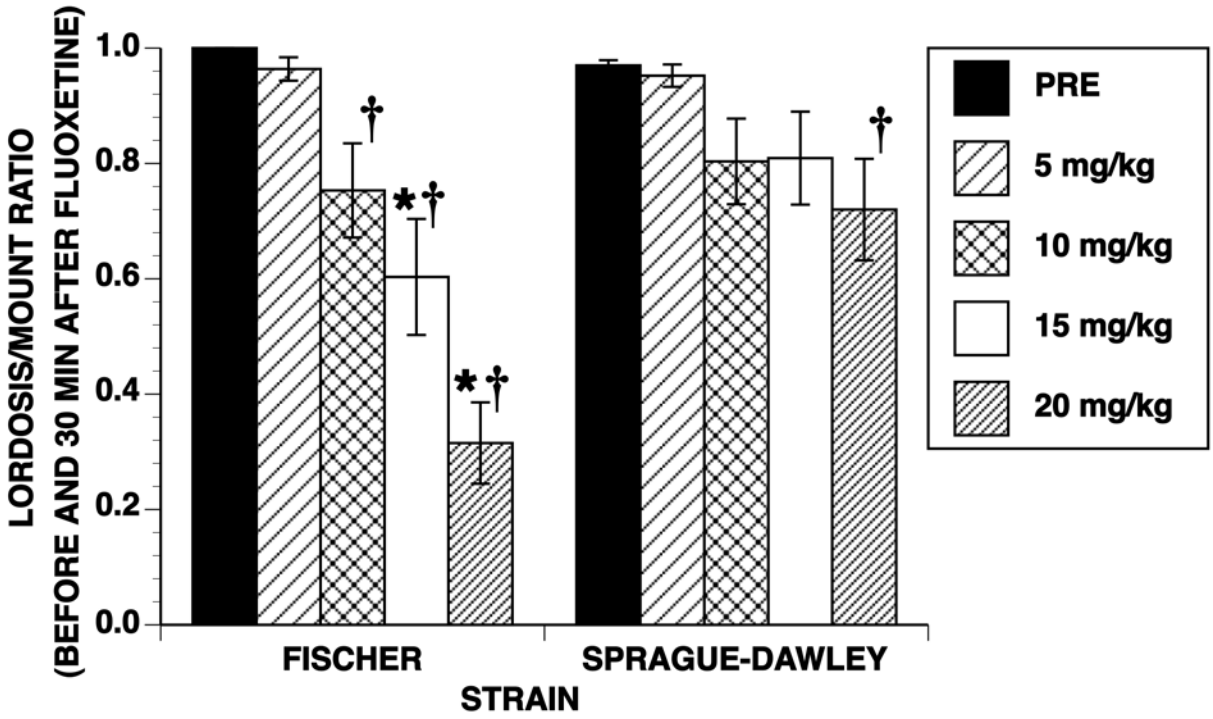

Figure 2.3 Fluoxetine dose-dependently decreased L/M ratios in hormonally primed, ovariectomized rats. Ovariectomized rats, hormonally primed with 0.067 µg EB/g body weight and 3.33 µg P/g body weight were pretested for sexual behavior 4 to 6 hr after P. Rats were then injected with 5, 10, 15, or 20 mg/kg of fluoxetine. Thirty minutes later, rats were again tested for 15 consecutive min. Data are the mean ± SE L/M ratios for the pretest (PRE) and 15 min after fluoxetine. The Ns for Fischer (F) and Sprague-Dawley (S-D) rats for 5, 10, 15 and 20 mg/kg fluoxetine, respectively, were 8, 8, 9 and 7 and 8, 7, 7 and 8. * indicates significant strain differences within dose of fluoxetine. † indicates significant difference from the pretest, within strain.

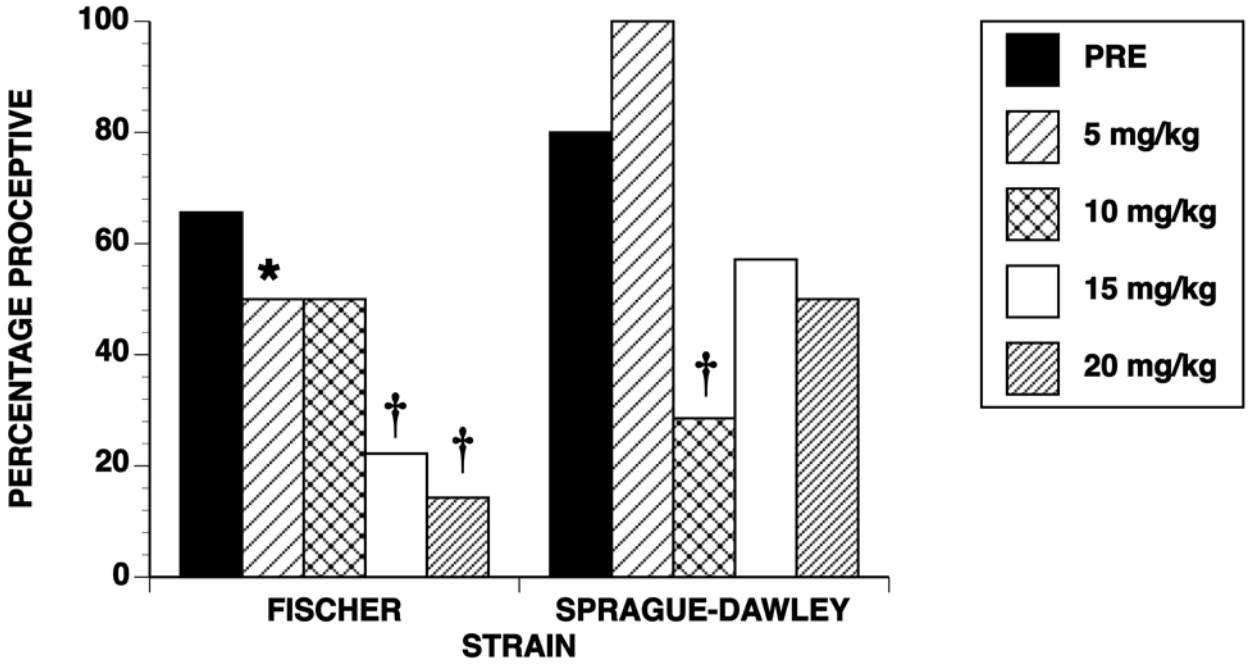

Figure 2.4 Dose-dependent effects of fluoxetine on proceptivity in hormonally primed, ovariectomized rats. Data are for the same rats shown in Figure 2.3 and are the percentage of rats that showed evidence of proceptivity before (PRE) and after fluoxetine treatment. * indicates significant strain differences within dose of fluoxetine. † indicates significant difference from the pretest, within strain.

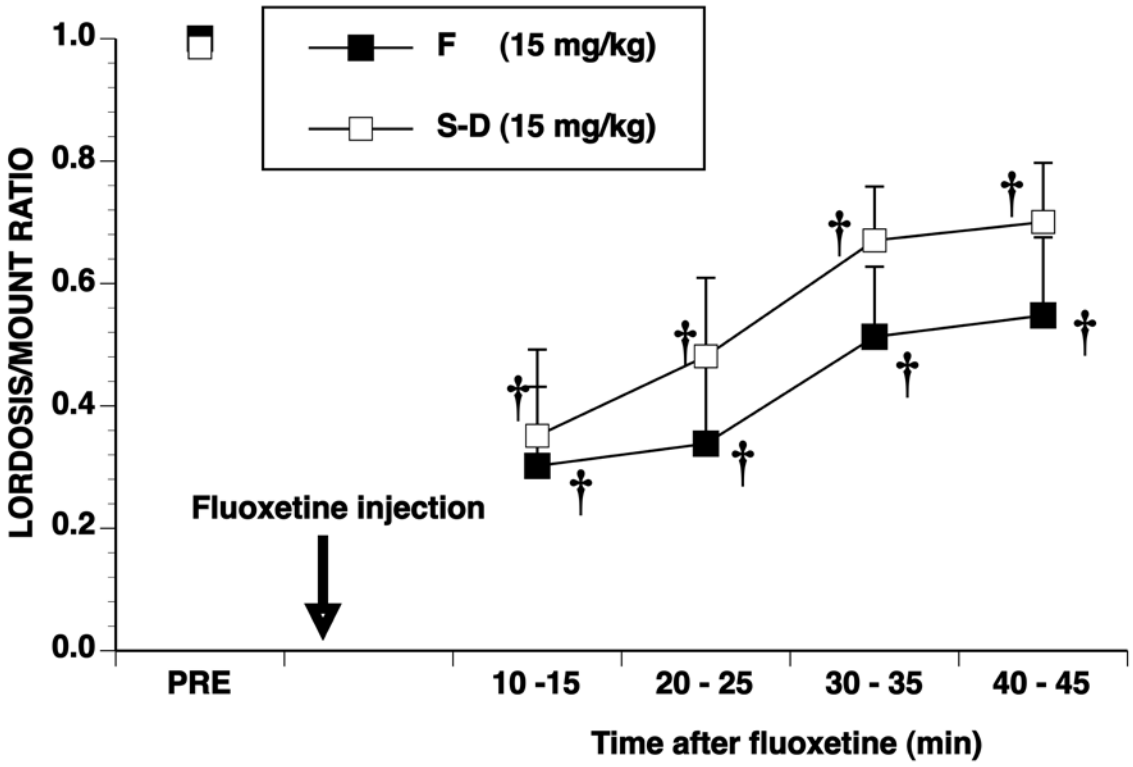

Figure 2.5 Rapid effects of fluoxetine on lordosis behavior.

Ovariectomized rats, hormonally primed with 0.067 µg EB/g body weight and 3.33 µg P/g body weight were pretested for sexual behavior 4 to 6 hr after P. Rats were then injected with 15 mg/kg of fluoxetine and 5 min later were tested for 40 consecutive min. Data are the mean ± SE L/M ratios for the pretest (PRE) and consecutive 10-min intervals initiated 5 min after fluoxetine injection. The Ns for Fischer (F) and Sprague-Dawley (S-D) rats respectively were 12 and 7. † indicates significant difference from the pretest, within strain.

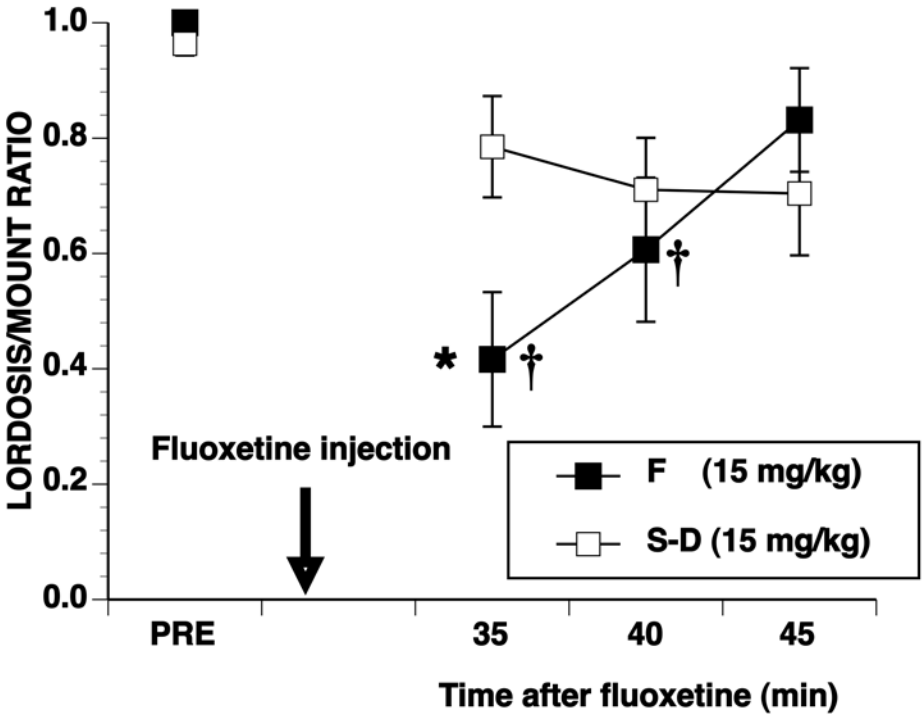

Figure 2.6 Strain differences when given constant hormonal priming. Ovariectomized rats, hormonally primed with 10 µg EB and 500 µg P per rat were pretested for sexual behavior 4 to 6 hr after P. Rats were then injected with 15 mg/kg of fluoxetine and 30 min later were tested for 15 consecutive min. Data are the mean ± SE L/M ratios for the pretest (PRE) and consecutive 5-min intervals 30 min after fluoxetine injection. The Ns for Fischer (F) and Sprague-Dawley (S-D) rats respectively were 11 and 11. * indicates significant strain differences within dose of fluoxetine. † indicates significant difference from the pretest, within strain.

Table 2.1 Early effects of fluoxetine on the percentage of females showing motor disturbance and proceptivity

	PRETEST	**10-15 MIN**	**20-25 MIN**	**30-35 MIN**	**40-45 MIN**
MOTOR DISTURBANCE					
Fischer	0	38.4	30.7	15.3	7.6
Sprague-Dawley	0	63.6	45.5	36.4	27.3
PROCEPTIVITY					
Fischer	76.9	18.18	0*	23.07	8.3*
Sprague-Dawley	100	20	36.36	45.45	54.45

*Indicates significant difference between strains, within time interval.

CHAPTER III

COMPARISON OF FEMALE FISCHER AND SPRAGUE-DAWLEY RATS IN THE RESPONSE TO KETANSERIN

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Chandra Suma Johnson Miryala, Cindy Hiegel, and Lynda Uphouse

ABSTRACT

The effect of the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, on lordosis behavior was examined in hormonally primed, ovariectomized Fischer and Sprague-Dawley females. Rats were primed with 0.067 µg/g body weight estradiol benzoate and 3.33 µg/g body weight progesterone. After a pretest for sexual behavior, rats were injected with 0.416 to 10 mg/kg ketanserin. In both strains, lordosis behavior, lordosis quality, and proceptivity were significantly reduced by ketanserin. There was modest evidence of a strain difference with Sprague-Dawley females slightly more sensitive to ketanserin. In a second experiment, the effects of 10 mg/kg fluoxetine, 1 mg/kg ketanserin, and their combination were examined to determine if the two drugs would have additive effects on sexual behavior. There was no evidence that the drugs were additive in their effect and

the strains did not differ in their response to the combined treatment. These findings are discussed in relation to prior evidence for strain differences in the sexual behavioral response to fluoxetine and to a receptor agonist acting preferentially at 5-HT_{1A} receptors.

Key Words: Rat strains, ovariectomized, fluoxetine, 5-HT_2 receptors, lordosis behavior, proceptivity

1. Introduction

A role for serotonin (5-HT) in the modulation of female rat sexual behavior is widely recognized (Mendelson and Gorzalka, 1990; Uphouse and Guptarak, 2010). A variety of drugs that increase extracellular 5-HT inhibit lordosis behavior but, depending on the receptor subtype activated, 5-HT receptor agonists can either inhibit or facilitate the behavior (Gonzalez et al., 1997; Hunter et al., 1985; Uphouse et al., 1996; Uphouse and Caldarola-Pastuszka, 1993; Wolf et al., 1998). The best characterized such agonists are the 5-HT_{1A} receptor agonists which rapidly inhibit lordosis behavior (Mendelson, 1992; Uphouse, 2000). As a result, it has been generally assumed that increased extracellular 5-HT reduces lordosis behavior by activation of 5-HT_{1A} receptors. In contrast, agonists that act primarily on 5-HT_2 or 5-HT_3 receptors facilitate lordosis behavior in female rats with relatively low sexual receptivity (Mendelson and Gorzalka, 1985; Wolf et al., 1999; Wolf et al., 1998). A potentially beneficial effect of 5-HT_2 and 5-HT_3 receptors is inferred from observations that 5-HT_2 and 5-HT_3 receptor agonists protect against the lordosis-inhibiting effects of 5-HT_{1A} receptor agonists (Maswood et al., 1998; Uphouse et al., 1994) and that 5-HT_2 and 5-HT_3 receptor antagonists inhibit lordosis behavior (Gonzalez et al., 1997; Maswood et al., 1997). Therefore, drugs which lead to generalized increases in extracellular 5-HT could disrupt the balance between activation of 5-HT receptors that inhibit and those that facilitate lordosis behavior. The relevance of such a disruption is evidenced by the large number of human females who show sexual dysfunction following treatment with selective serotonin reuptake inhibitors

(SSRIs), such as fluoxetine (Clayton et al., 2006; Clayton, 2002; Gelenberg et al., 2000; Gregorian et al., 2002).

SSRIs block the serotonin transporter (SERT) and thereby lead to an increase in extracellular 5-HT and enhanced activation of all 5-HT receptors (Fuller et al., 1991; Gobert et al., 1997; Perry and Fuller, 1992, 1993; Sghendo and Mifsud, 2011; Tao et al., 2002; Tavoulari et al., 2009), but it is the activation of 5-HT_{1A} receptors that has been postulated to account for the lordosis inhibition that follows treatment with fluoxetine (Guptarak et al., 2010). However, not all rat strains show comparable vulnerability to the lordosis-inhibiting effects of either fluoxetine or a 5-HT_{1A} receptor agonist (Miryala et al., 2013; Uphouse et al., 2002). For example, Fischer rats have a higher baseline level of 5-HT than Sprague-Dawley rats (Rosecrans et al., 1986), show an accentuated 5-HT response to stress (Dhabhar et al., 1993; Kosten and Ambrosio, 2002; Rosecrans et al., 1986), and are more responsive to the lordosis-inhibiting effects of fluoxetine than are Sprague-Dawley females (Maswood et al., 2008; Miryala et al., 2013; Uphouse et al., 2006). However, Fischer females are less responsive than Sprague-Dawley females to the lordosis-inhibiting effects of a 5-HT_{1A} receptor agonist (Uphouse et al., 2002) suggesting that strain differences in the lordosis response to fluoxetine may include additional 5-HT receptors. To date, there have been no studies of a potential rat strain difference in the response to either 5-HT_2 or 5-HT_3 receptor active compounds. Therefore, in the following experiment, a potential strain difference in the sexual behavioral response to the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, was examined. Emphasis was placed on the 5-$HT_{2A/2C}$ receptor because this receptor has been most

thoroughly investigated for it's impact on the response to 5-HT_{1A} receptor agonists (Uphouse and Guptarak, 2010) and because such antagonists can augment the effects of SSRIs (Boothman et al., 2006; Cremers et al., 2004; Marek et al., 2003; Marek et al., 2005). Such augmentation is thought to result from the ability of 5-$HT_{2A/2C}$ receptor antagonists to increase firing of 5-HT neurons in the dorsal raphe nucleus (DRN) (Boothman et al., 2003; Boothman and Sharp, 2005; Cremers et al., 2004). Therefore, 5-$HT_{2A/2C}$ receptor antagonists, by increasing extracellular 5-HT in lordosis-controlling brain areas, would be expected to reduce lordosis behavior both by increasing activation of inhibitory 5-HT_{1A} receptors and by antagonizing any protective effect of 5-$HT_{2A/2C}$ receptors. Because of the greater sensitivity of Sprague-Dawley rats to the lordosis-inhibiting effect of a 5-HT_{1A} receptor agonist but lesser sensitivity to the SSRI, fluoxetine, it was hypothesized that Sprague-Dawley females might show a greater sensitivity to antagonism of 5-$HT_{2A/2C}$ receptors.

2. Methods

2.1 Materials

Estradiol benzoate (EB), progesterone (P), sesame seed oil, the selective serotonin reuptake inhibitor (SSRI), fluoxetine (methyl [3-phenyl-3-4-(trifluoromethyl)-phenoxy] propyl] ammonium chloride), and the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin (3-[2-[4-(4-fluorbenzoyl)-1-piperdinyl]ethyl]- 2,4(1H,3H)-quinazolinedione), were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Isoflurane (AErrane®) and suture materials were purchased from Butler Schein Animal Health (Dublin, OH). Food

(Rodent Lab Diet 5001) was purchased from Lab Animal Supply (Highland Village, TX, USA). All other supplies were purchased from Fisher Scientific (Houston, TX, USA).

2.2 Animals, housing and surgical procedures

Female Fischer and Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA) and housed 2 per cage in standard shoebox caging (45.72 × 24.13 × 20.59 cm). The housing area was maintained at 25° C and 65% humidity with lights on from 12:00 midnight to 12:00 noon. Animals varied in their age within experiments but were always matched between Fischer and Sprague-Dawley females and counterbalanced between treatment conditions. Food and water were available *ad libitum.* Rats were ovariectomized under AErrane® anesthesia as described previously (White and Uphouse, 2004) approximately 2 weeks after their arrival. Hormonal priming was with EB followed 48 hours later with P and occurred 10 to 14 days after ovariectomy. Hormones were dissolved in sesame seed oil and injected subcutaneously. Since Fischer and Sprague-Dawley females differ in their body weight, hormones were administered per body weight (0.067 µg/g EB and 3.333 µg/g P). At the time of behavioral testing, in the first experiment, females were 12 to 22 weeks old; the mean ± standard error (S.E.) body weights of Fischer and Sprague-Dawley were 162.7 ± 1.55 and 273.0 ± 4.15, respectively; in the second experiment, females were 13 to 17 weeks old with mean ± S.E. body weights of Fischer and Sprague-Dawley rats of 166.6 ± 2.05 and 264.2 ± 3.85, respectively. All procedures were in accordance with the NIH Guide for the Care and Use of Animals in Research and were approved by the Institutional Animal Care and Use Committee at Texas Woman's University.

2.3 Treatment of animals

On the day of testing, 4 to 6 hr after P injection, rats were pretested for sexual behavior as previously described (White and Uphouse, 2004). Rats were placed into the home cage of a sexually active male and behavior was monitored until 10 mounts had occurred or for a maximum of 10 min. In the first experiment, immediately following the pretest, females were injected intraperitoneally (ip) with deionized water (DI) or with 0.416, 0.5, 0.75, 1.0, 2.0, 5.0 or 10.0 mg/kg of the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, in a volume of 1 ml/kg. Behavioral testing began immediately after the ketanserin injection and was recorded for 30 consecutive min. Data were grouped into the first and second 15 min intervals for statistical analysis. In the second experiment, immediately following the pretest, females were injected with fluoxetine (10 mg/kg; volume of 1 ml/kg) or deionized water (DI). Fifteen min later, DI treated rats were injected intraperitoneally with 1 mg/kg ketanserin; fluoxetine-treated rats were injected with ketanserin (1 mg/kg) or DI. This led to three groups: fluoxetine only, ketanserin only, and the combination of fluoxetine and ketanserin. Fifteen min after the ketanserin or DI treatment, females were placed into the male's cage for 15 min of sexual behavioral testing. Data for the 15 min interval were grouped for statistical comparison.

2.4 Behavioral testing procedures

The lordosis/mount (L/M) ratio (number of lordosis reflexes by the female divided by the number of mounts by the male) and lordosis quality scores (on a scale of 1 to 4) were measured as previously described (White and Uphouse, 2004). Lordosis quality was calculated as the sum of the individual lordosis quality scores divided by the

number of lordosis responses. Proceptivity was measured by the presence or absence of hopping and darting behavior. L/M ratios, lordosis quality scores, and mounts were recorded for the pretest and for the 15-min test intervals. Proceptive behavior was recorded as present or absent for the pretest and the entire test interval.

2.5 Statistical procedures

For the first experiment, L/M ratios, lordosis quality and number of mounts were analyzed by repeated measures analysis of variance (ANOVA) with strain and dose of ketanserin as independent factors and with first or second testing interval as the repeated factor. L/M ratios were subjected to regression on dose for estimation of the IC_{50}. IC_{50} (dose leading to 50% of maximum effect) was defined as an L/M ratio of 0.5. For experiment 2, strain and treatment condition were independent factors and time relative to pretest was the repeated factor. Proceptivity data were analyzed using Chi-Square and Fischer's Exact Test procedures. Data were analyzed with SPSS version 19.0 (for PC) and the statistical reference was Zar (2010). Post-hoc Tukey comparisons were computed manually and an alpha of 0.05 was required for statistical significance.

3. Results

The effects of ketanserin on lordosis behavior of Fischer and Sprague-Dawley females are shown in Figure 3.1. There was a significant effect of strain ($F_{1,109} = 5.14$, $p \leq 0.025$) and dose of ketanserin ($F_{7, 109} = 11.47$, $p \leq 0.001$). The strain difference reflected a lower L/M ratio of Sprague-Dawley than Fischer females that was most evident during the second 15 min interval after ketanserin treatment. In comparison to

the DI water control, Sprague-Dawley females showed a significant decline in the L/M ratio at lower doses of ketanserin than were evident in Fischer females. However, the IC_{50} for the two strains were relatively similar. For the first 15 min interval, the IC_{50} for Fischer and Sprague-Dawley, respectively, was 12.73 and 10.62 mg/kg. For the second 15 min interval, the IC_{50} for Fischer and Sprague-Dawley, respectively, were 6.12 and 7.5 mg/kg. There was a significant difference between the first and second 15 min interval (ANOVA for time after ketanserin, $F_{1,109} = 39.93$, $p \leq 0.001$), but there was no interaction between time and strain ($p > 0.05$) and none of the interactions with strain were significant (all $p > 0.05$).

The effects of ketanserin on lordosis quality were similar to those for L/M ratios and were also more obvious during the second 15 min interval (ANOVA for time, $F_{1,106} = 9.27$, $p \leq 0.003$, see Figure 3.2). There was a significant effect of the dose of ketanserin on lordosis quality ($F_{7,106}= 4.14$, $p \leq 0.001$) and, as for L/M ratios, quality scores were slightly more likely to be affected in Sprague-Dawley than in Fischer females (ANOVA for strain, $F_{1,106} = 9.21$, $p \leq 0.003$). Nevertheless, none of the interactions with strain or dose were significant (all $p > 0.05$).

Fischer and Sprague-Dawley females received comparable mounting from the males. The only significant effect was between strain and time ($F_{1,110} = 5.72$, $p \leq 0.018$) that reflected a decline between the first and second 15 min intervals in number of mounts for Fischer, but not Sprague-Dawley rats. For Fischer rats, the means ± S.E. numbers of mounts for the 15 and 30 min intervals, respectively, were 20.5 ± 0.97 and

17.8 ± 0.98; for Sprague-Dawley females, the means ± S.E. were 19.2 ± 0.98 and 19.8 ± 0.99, respectively.

Prior to injection, a greater proportion of Sprague-Dawley than Fischer females showed evidence of proceptivity (for Fischer and Sprague-Dawley, respectively, 61.9% and 80.9%; Fisher Exact test, $p \leq 0.029$). After injection, there was a significant effect of the treatment in Fischer rats (Chi-Square = 20.35, df = 7, $p \leq 0.005$) but this reflected a decrease in proceptivity relative to the DI control. When only ketanserin groups were compared, there was no dose effect ($p > 0.05$). There was no effect of treatment on proceptivity of Sprague-Dawley females (Chi-Square = 9.3, df = 7, $p > 0.05$) (Figure 3.3). However, when groups were collapsed across the dose of ketanserin and compared to the DI control, proceptivity of ketanserin-treated females was significantly reduced in both strains (see Figure 3 inset; for Fischer and Sprague-Dawley, respectively, Chi-Square = 13.08, $p \leq 0.001$ and Chi-Square = 4.93, $p \leq 0.026$).

The second experiment was designed to determine if fluoxetine and ketanserin would amplify the effects of either drug alone. A dose of 10 mg/kg fluoxetine (Miryala et al., 2013) and 1 mg/kg ketanserin (Experiment 1) were chosen and the effects of either drug or their combination were compared. For L/M ratios, there was a significant effect of the type of treatment ($F_{2, 45} = 4.43$, $p \leq 0.017$), time ($F_{1, 45} = 90.71$, $p \leq 0.0001$) and their interaction ($F_{2, 45} = 3.39$, $p \leq 0.042$) (Figure 3.4). Neither strain nor any interactions with strain were significant. All treatment conditions reduced L/M ratios relative to their pretest ($q_{45, 2} = 2.85$, $p < 0.05$) but L/M ratios after fluoxetine injection were higher than in the other 2 groups. This was especially evident in Fischer females where fluoxetine-

treated rats had significantly higher L/M ratios than the other two treatment conditions (for ketanserin, alone, and both drugs, respectively, $q_{3, 45} = 3.65$ and 5.25, $p \leq 0.05$). For Sprague-Dawley females, L/M ratios of fluoxetine-treated rats did not differ significantly from either of the 2 other groups (Tukey's $q_{3, 45,}$ all $p > 0.05$). In neither strain did the L/M ratios of rats treated with both ketanserin and fluoxetine differ from L/M ratios after ketanserin, alone (all $p > 0.05$).

Two females (one from each strain) failed to show any lordosis behavior after treatment and were excluded from the analysis for lordosis quality. For the remaining rats, there was not a differential effect of treatment on lordosis quality ($p > 0.05$; see Table 3.1). Although there was a slight effect of strain ($F_{1, 43} = 5.50$, $p \leq 0.05$) and time ($F_{1, 43} = 18.42$, $p \leq 0.001$), lordosis quality remained relatively high throughout the testing period with the exception of Sprague-Dawley female given both drugs. For this treatment, in this strain, lordosis quality was significantly reduced relative to the pretest ($q_{3, 43} = 5.93$, $p \leq 0.05$) and the combination of the two drugs differed from both fluoxetine only and ketanserin only groups (respectively, $q_{3, 43} = 5.07$ and 5.33, $p \leq 0.05$).

As in the first experiment, more Sprague-Dawley than Fischer females showed proceptivity (34.6% of Fischer and 92% of Sprague-Dawley females were proceptive; Chi-Square = 17.95, df = 1, $p \leq 0.001$). Rats assigned to different treatment conditions did not differ in the pretest (Chi-Square for Fischer = 0.552 and Sprague-Dawley = 1.0,

$p > 0.05$) but there was a treatment-dependent effect on proceptivity after treatment (respectively for Fischer and Sprague-Dawley females, Chi-Square = 7.63 and 7.29, df = 2, $p \leq 0.03$) (Figure 3.5). For Sprague-Dawley females, the treatment effect

resulted from higher proceptivity in fluoxetine-treated rats compared to the other 2 groups (for ketanserin plus fluoxetine and ketanserin, alone, respectively, Chi-Square = 6.34 and 4.96, df = 1, $p \leq 0.04$). Although a similar pattern was present in Fischer females, because of the low overall proceptivity in Fischer rats, there were no treatment effects. Strain differences were present only in rats given fluoxetine (Chi-Square = 5.52, df = 1, $p \leq 0.04$) and reflected the lower overall proceptivity of Fischer females rather than a strain difference in response to the treatment.

There were no differences between strains or among treatments on the number of mounts received by the females. The mean ± S.E. number of mounts for Fischer rats for fluoxetine only, ketanserin only, and their combination, respectively, were 21.7 ± 2.6, 15.42 ± 2.31 and 14.7 ± 1.84; the average number of mounts for fluoxetine only, ketanserin only and their combination for Sprague-Dawley, respectively, were 14.4 ± 2.48, 16.7 ± 2.0, and 13.1 ± 1.76.

4. Discussion

The present study was designed to expand information about the strain differences in the 5-HT system which could potentially account for the lower sensitivity of Sprague-Dawley females to the lordosis inhibiting effects of fluoxetine but greater sensitivity to the lordosis-inhibiting 5-HT_{1A} receptor agonist, (+/-) 8-hydroxy-2- (di-n-propylamino) tetralin (8-OH-DPAT). Ketanserin reduced lordosis behavior in both strains and inhibition was greatest during the second 15 min test interval (15 to 30 min after injection), consistent with earlier studies showing time-dependent effects of ketanserin following infusion into the ventromedial nucleus of the hypothalamus (Sinclair-Worley

and Uphouse, 2004; Wolf et al., 1998). In comparison to robust strain differences in the lordosis-inhibitory effect of the 5-HT_{1A} receptor agonist, 8-OH-DPAT (Uphouse et al., 2002), strain differences in the response to the 5-$HT_{2A/2C}$ receptor antagonist were relatively modest and evident only during the 2nd 15 min test interval. Nevertheless, the IC_{50} was comparable in the two strains. However, that Sprague-Dawley females showed a slightly greater effect of ketanserin may indicate a greater dependency of this strain on the lordosis-facilitating effects of 5-$HT_{2A/2C}$ receptors.

Ketanserin's ability to reduce lordosis behavior is believed to result from its antagonism of 5-$HT_{2A/2C}$ receptors (Mendelson, 1992; Uphouse and Guptarak, 2010) but whether 5-HT_{2A} or 5-HT_{2C} receptors are responsible is unclear. Ketanserin blocks both 5-HT_{2A} and 5-HT_{2C} receptors but is reported to have the greater affinity for 5-HT_{2A} receptors (Hoyer et al., 2002). However, in the ventromedial hypothalamus (VMN), it is 5-HT_{2C} receptors that have been implicated in lordosis modulation (Wolf et al., 1999). In this brain area, the 5-$HT_{2B/2C}$ receptor antagonist, SB206553, but not the 5-HT_{2A} receptor antagonist, MDL 100,907, mimicked effects of ketanserin. In contrast, 5-HT_{2A} receptors may be responsible for 5-HT_2 receptor-mediated drug effects in the medial preoptic area (Gonzalez et al., 1997). Therefore, both 5-HT_{2A} and 5-HT_{2C} receptors and several brain areas may contribute to the decline in lordosis behavior after the systemic treatment with ketanserin.

Differences between Fischer and Sprague-Dawley rats have been repeatedly noted with considerable evidence for differential functioning of the 5-HT system (Burnet et al., 1994; Fernandez et al., 2003; Miryala et al., 2013; Rosecrans et al., 1986; Uphouse et al.,

2002). Of special relevance to the current study is evidence that Fischer rats may have more SERT in the DRN than other rat strains (Burnet et al., 1996; Fernandez et al., 2003). If so, relative to Sprague-Dawley females, Fischer rats would be expected to have less extracellular 5-HT in the DRN following treatment with the SERT blocker, fluoxetine. With less consequent activation of the somatodendritic auto inhibitory 5-HT_{1A} receptors, Fischer females might show a greater elevation of extracellular 5-HT in lordosis-relevant brain areas such as the VMN following treatment with SSRIs. With fluoxetine's additional antagonism at 5-HT_2 receptors (Jenck et al., 1993; Palvimaki et al., 1996), the increase in 5-HT release would disrupt the balance between the lordosis-inhibiting (5-HT_{1A}) and lordosis-facilitating (5-HT_{2C}) receptors to a greater extent in Fischer than in Sprague-Dawley females.

In contrast, following treatment with a 5-HT_{1A} receptor agonist, the higher midbrain SERT of Fischer females would not impact the strain response since the agonist would directly activate lordosis-inhibiting 5-HT_{1A} receptors to reveal a greater sensitivity of Sprague-Dawley females to the 5-HT_{1A} receptor agonist.

The current studies offer indirect evidence that strain differences between Fischer and Sprague-Dawley females do not include 5-HT_2 receptors. 5-HT_2 receptors in the midbrain decrease firing of DRN neurons possibly by activation of inhibitory GABA neurons (Boothman et al., 2003; Boothman and Sharp, 2005) and 5-HT_2 receptor antagonists increase release of 5-HT in areas terminal to DRN neurons (Boothman et al., 2006). In the current study, blocking 5-HT_2 receptors with ketanserin would be expected to increase DRN firing and release of 5-HT and thereby increase activation of 5-HT_{1A}

receptors and reduce activation of 5-HT_2 receptors in lordosis-relevant brain areas. It was, therefore, expected that Sprague-Dawley rats would show greater sensitivity to ketanserin, but this was not the case. In the absence of a remarkable strain difference in the response to 5-HT_2 receptor antagonism, the resulting disruption of balance between the inhibitory and facilitatory 5-HT_2 receptors would be substantial in both strains but would produce a minimal strain difference, as observed in the current study.

Since fluoxetine not only blocks SERT but also blocks 5-HT_2 receptors (Jenck et al., 1993; Palvimaki et al., 1996), it was anticipated that ketanserin plus fluoxetine would be additive in their effect on lordosis behavior. Such an accentuated response in hippocampal extracellular 5-HT has been reported in Fischer females following treatment with another SSRI, citalopram, but not following fluoxetine (Fernandez et al., 2003). However, such an augmentation following fluoxetine has been reported by other investigators (Cremers et al., 2004). Nevertheless, in the current study, fluoxetine and ketanserin were not additive in their effect on lordosis behavior and there was no evidence of a strain difference.

Therefore, the current studies provide evidence that previously reported difference between Fischer and Sprague-Dawley females in response to fluoxetine or a 5-HT_{1A} receptor agonist are unlikely to depend on a strain difference in 5-HT_2 receptor action. However, since ketanserin blocks both 5-HT_{2A} and 5-HT_{2C} receptors, more selective 5-HT_2 receptor antagonists are needed to definitively eliminate 5-HT_2 receptors from consideration. Moreover, only a single dose of fluoxetine and ketanserin were examined in the current study. Additional dose combinations would be useful to completely rule

out such a strain difference in 5-HT_2 receptors and/or additive/synergistic effects of 5-HT_2 receptor antagonists and fluoxetine. Finally, it is important to note that ketanserin also blocks alpha-1 adrenergic receptors (Hoyer et al., 1987; Marwood, 1994) so that we cannot rule out possible strain differences in these receptors that could have masked the existence of a strain difference for 5-HT_2 receptor antagonism.

5. Conclusions

In summary, ketanserin dose-dependently inhibited lordosis behavior in both Fischer and Sprague-Dawley females. Strain differences, though present, were modest and are more likely to reflect the greater sensitivity of Sprague-Dawley females to 5-HT_{1A} receptor activation than a strain difference in 5-HT_2 receptor functioning. In addition, ketanserin and fluoxetine were not additive in their effect on lordosis, and the combined treatment reflected primarily the lordosis inhibition with ketanserin alone.

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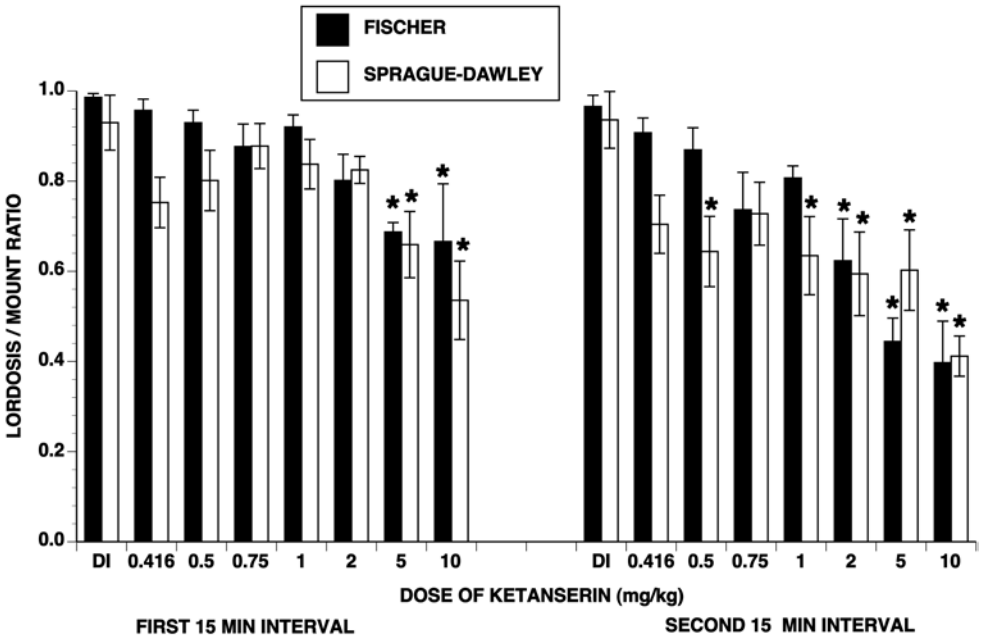

Fig. 3.1. L/M ratio after varying doses of ketanserin treatment.

Hormonally primed, ovariectomized rats were pretested for sexual behavior 4 to 6 hr after progesterone. Rats were then injected with deionized (DI) water or with 0.416, 0.5, 0.75, 1, 2, 5 or 10 mg/kg of ketanserin. Fifteen minutes later, rats were again tested for two consecutive 15 min intervals. Data are the mean ± SE L/M ratios for the two 15 min interval after injection with deionized water or ketanserin. Pretest L/M ratios (0.99 ± 0.003 and 0.98 ± 0.007, respectively, for Fischer and Sprague-Dawley females) did not differ. The Ns for Fischer rats for (DI) water or 0.416, 0.5, 0.75, 1, 2, 5 or 10 mg/kg of ketanserin, respectively, were 5, 8, 9, 6, 10, 5, 13 and 7. Ns for Sprague-Dawley rats, respectively, were 5, 8, 10, 6, 9, 5, 14 and 6. *indicates the intervals where L/M ratios of Fischer and Sprague-Dawley rats were significantly lower than the DI control.

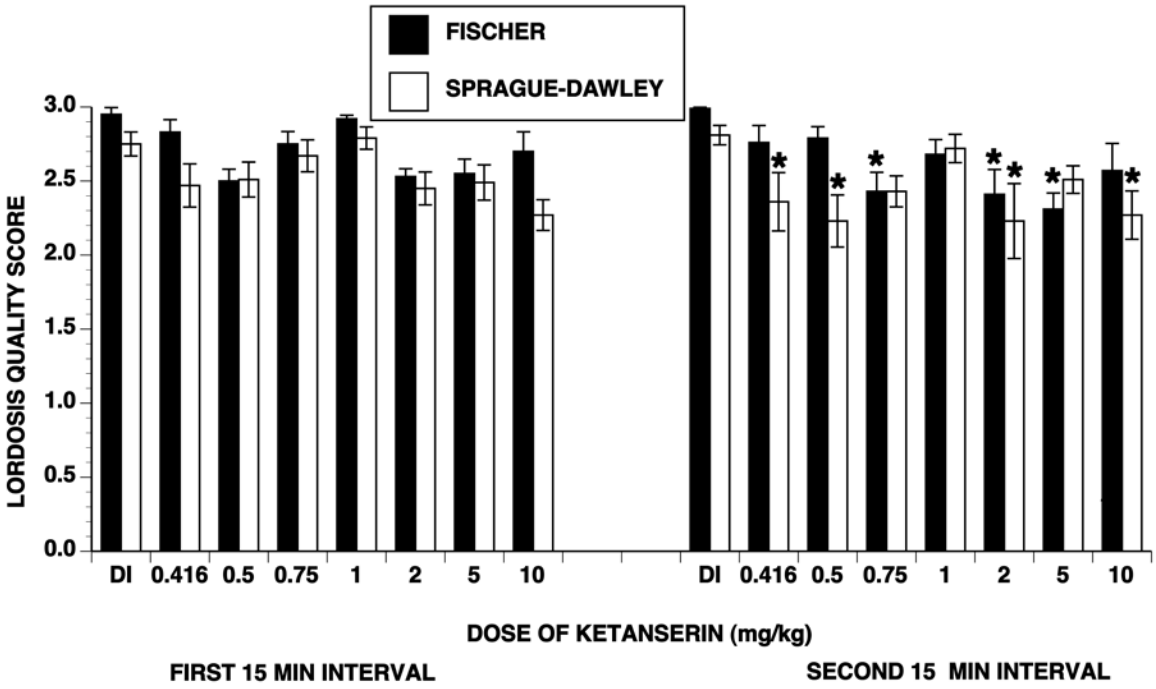

Fig. 3.2. Lordosis quality after ketanserin treatment.

Data are the mean ± SE lordosis quality score for the pretest and two 15 intervals after treatment with ketanserin or deionized water (DI) and are for the same rats as in Figure 3.1. The Ns for Fischer rats for DI water or 0.416, 0.5, 0.75, 1, 2, 5 or 10 mg/kg of ketanserin, respectively, were 5, 8, 9, 6, 10, 5, 13 and 7. Ns for Sprague-Dawley rats, respectively, were 5, 8, 10, 6, 9, 5, 14 and 6. *indicates the intervals where lordosis quality of Fischer and Sprague-Dawley rats were significantly lower than the DI control.

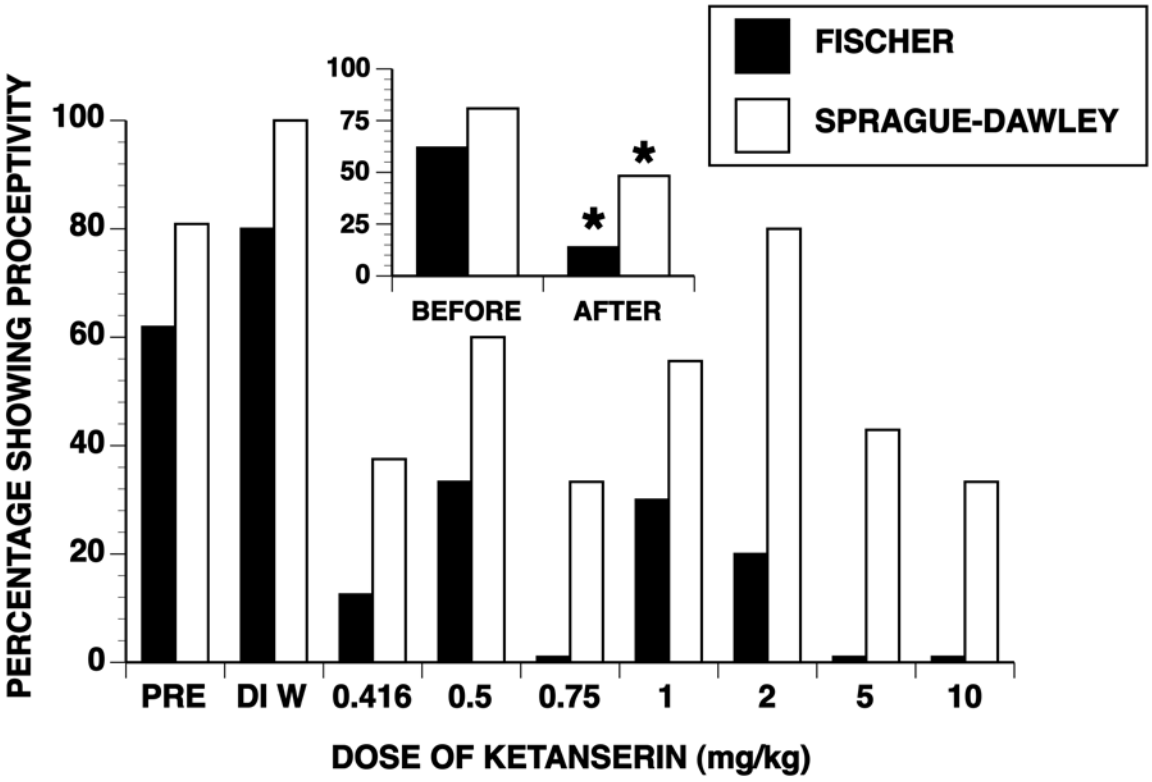

Fig. 3.3. Effects of ketanserin on proceptivity.

Data are the percentage of rats showing proceptivity after the various doses of ketanserin treatment and for the same rats as in Figure 3.1. The inset shows the percentage of rats when collapsed across doses of ketanserin. *indicates significant decline in proceptivity relative to the DI control, within strain.

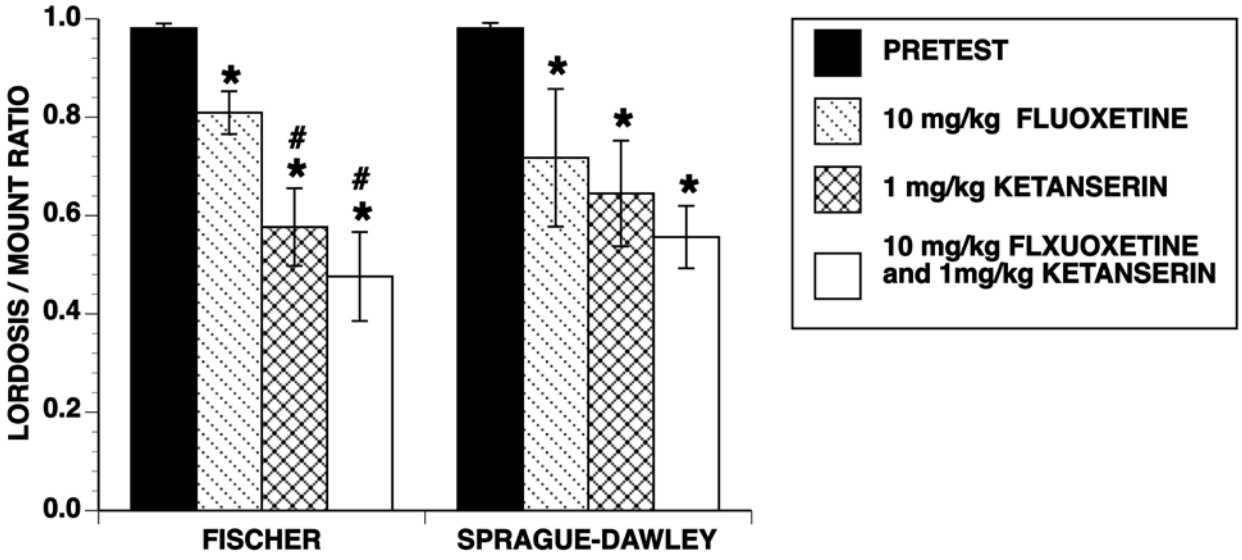

Fig. 3.4. Ketanserin and fluoxetine are not additive for lordosis inhibition. Hormonally primed, ovariectomized rats were pretested for sexual behavior 4 to 6 hr after progesterone. Rats were then injected with 10 mg/kg fluoxetine or deionized water (DI). Fifteen minutes later DI injected rats were injected with 1 mg/kg ketanserin; fluoxetine-treated rats were injected with 1 mg/kg ketanserin or DI. This led to three groups, fluoxetine only, ketanserin only, fluoxetine and ketanserin. Fifteen minutes later, rats were again tested for 15 consecutive min. Data are the mean ± SE L/M ratios for the pretest and 15 consecutive min after injection. The Ns for Fischer rats given fluoxetine, ketanserin, or both drugs were 8, 7 and 11. Ns for Sprague-Dawley rats given fluoxetine, ketanserin, or both drugs were 7, 7 and 11. *indicates significant difference from the pretest within treatment, # indicates a difference from fluoxetine within strain.

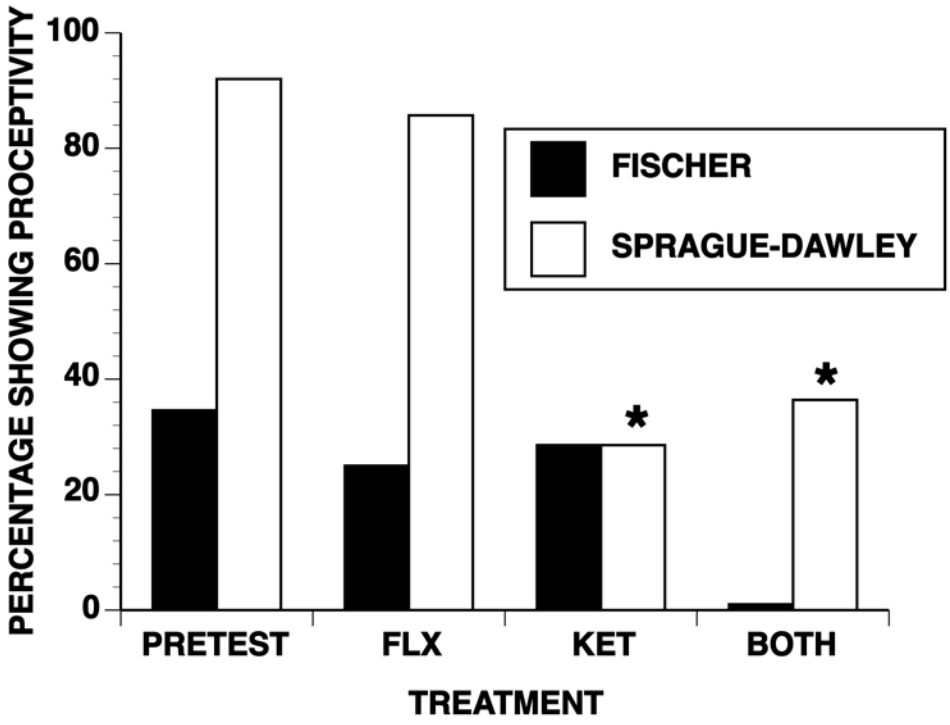

Fig. 3.5. Effects of fluoxetine (FLX), ketanserin (KET), and their combination (BOTH) on proceptivity.

Data are the percentage of rats showing proceptivity after the 3 treatments and are for the same rats as in Figure 3.4. Data are for the Pretest and the 15 min interval after treatment. *indicates a significant difference from both the pretest and fluoxetine, within strain.

Table 3.1

Treatment effects on lordosis quality scores

	Treatment			
Strain	**Pretest**	**Fluoxetine 10 mg/kg**	**Ketanserin 1 mg/kg**	**Fluoxetine + Ketanserin**
Fischer	2.8 ± 0.04	2.9 ± 0.04	2.8 ± 0.07	2.8 ± 0.07
Sprague-Dawley	2.8 ± 0.05	2.7 ± 0.12	2.7 ± 0.13	2.3 ± 0.19*#

*****Indicates significantly different from Fischer within treatment
#Indicates significantly different from all other groups within strain

CHAPTER IV

DISCUSSION

With the discovery of fluoxetine and its efficacy for treatment of depression, the antidepressant has been widely used not only for depression but for other mood disorders of greater prevalence in females (Hashimoto, 2010; Nazari et al., 2013). However, in spite of its efficacy for these mood disorders, fluoxetine causes sexual dysfunction in a large proportion of patients and the onset of sexual dysfunction precedes the clinical manifestations of drug efficacy for depression. As a consequence, sexual dysfunction is a major factor in patient noncompliance (Michelson et al., 2000). However, not all patients exhibit such sexual dysfunction. The main objective of this study was to provide information as to why some females experience severe sexual dysfunction following treatment with fluoxetine while others show minimal, if any, effects. Specifically, the current studies were designed to determine if Fischer and Sprague-Dawley female rats differed in their sexual behavioral response after an acute treatment with fluoxetine. The major outcomes of this study were: (i) consistent with prior studies, fluoxetine reduced female rat sexual behavior in both hormonally-primed, ovariectomized and in naturally-cycling rats; (ii) hormonally primed, ovariectomized rats were more sensitive to the lordosis-inhibiting effects of fluoxetine than the intact, naturally cycling females; (iii) in both hormonally-primed and naturally cycling rats, Sprague-Dawley females were less

sensitive to the lordosis-inhibiting effects of fluoxetine than Fischer females; (iv) a 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, reduced lordosis behavior in both strains with a slightly greater effect in Sprague-Dawley females; but the difference was modest in comparison to the strain difference in response to fluoxetine; and (v) the combination of fluoxetine and ketanserin did not amplify negative effects on lordosis behavior relative to the individual drugs alone.

Since recognition of the sexual side effects of fluoxetine, there has been considerable discussion about the responsible mechanisms with most emphasis placed on the 5-HT system (Haenisch and Bönisch, 2011). Fluoxetine increases extracellular 5-HT by blocking the SERT and thereby has the potential to increase activation of all 5-HT receptors (Malagié et al., 1995; Tao et al., 2002). Regarding the sexual side effects in female rats, activation of 5-HT_{1A} receptors is thought to contribute to the sexual dysfunction following fluoxetine treatment (Guptarak et al., 2010). Because of the greater sensitivity of Sprague-Dawley females to 5-HT_{1A} receptor activation, it was hypothesized that Sprague-Dawley females might be more sensitive to fluoxetine, but this was not the case. Fischer females were more sensitive to the lordosis-inhibiting effect of fluoxetine than Sprague-Dawley females and this was evident in both hormonally-primed and naturally cycling rats. The greater sensitivity of Fischer females to the acute effect of fluoxetine is consistent with prior studies demonstrating a greater effect of repeated fluoxetine on estrous cyclicity in this strain (Maswood et al., 2008; Uphouse et al., 2006).

Since, in the current experiment with ovariectomized females, hormonal treatment was administered according to body weight, the heavier Sprague-Dawley females

received a greater absolute amount of hormones. This could have been a confounding factor in the strain difference since EB and P can alter the number and function of 5-HT_1 and 5-HT_2 receptors (Sumner et al., 1999; Moses et al., 2000; Mize et al., 2003) and progesterone has been reported to reduce the lordosis inhibiting effects of fluoxetine (Guptarak et al., 2010). However, we do not believe this to be the case since strain differences remained even when rats were administered the identical hormonal treatment. Moreover, differential exogenous hormonal treatment cannot account for the comparable strain difference in naturally cycling females.

Studies with ketanserin were initiated because of the apparent inconsistency between the strain difference in response to fluoxetine and the 5-HT_{1A} receptor agonist. 5-$HT_{2A/2C}$ receptor agonists can protect against the lordosis-inhibiting effects of 5-HT_{1A} receptor activation (Maswood et al., 1996) so it was hypothesized that, following treatment with fluoxetine, 5-$HT_{2A/2C}$ receptors might be providing greater protection in Sprague-Dawley than in Fischer females. Since such strain differences did not exist, 5-$HT_{2A/2C}$ receptors are unlikely to explain the apparent inconsistency. Therefore, the most viable explanation for the directional reversal in response to fluoxetine and a 5-HT_{1A} receptor appears to reside in a strain difference in levels of 5-HT at neuronal sites important for lordosis behavior.

Fischer rats have been reported to have more SERT mRNA in the dorsal raphe nucleus (DRN) than Sprague-Dawley rats (Burnet et al., 1994). If there is greater SERT activity in the dorsal raphe of Fischer females, fluoxetine should produce a smaller increase in extracellular 5-HT in that brain area. With such a smaller increase in

extracellular 5-HT, there would be a correspondingly smaller increase in activation of DRN inhibitory 5-HT_{1A} autoreceptors. As a consequence, fluoxetine's reduction in the firing of DRN 5-HT neurons would be smaller in Fischer than in Sprague-Dawley rats. This would result in more extracellular 5-HT after fluoxetine in Fischer females than in Sprague-Dawley females in areas of the brain such as the mediobasal hypothalamus that are important for sexual behavior. Such an explanation would allow for Fischer rats to be more sensitive to fluoxetine and also less sensitive to a 5-HT_{1A} receptor agonist.

Although the current studies appear to rule out 5-$HT_{2A/2C}$ receptors as a contributor to the strain difference in response to fluoxetine, it is important to note that ketanserin has both 5-HT_{2A} and 5-HT_{2C} receptor blocking activity (Kristiansen and Dahl, 1996) so additional studies with more selective antagonists are required before definitely ruling out a strain difference in these receptors. In addition, ketanserin binds to both alpha - adrenergic and histamine receptors (Hoyer et al., 1987; Marin et al., 1990; Marwood, 1994; Wouters et al., 1985) which may have (a) influenced the response to ketanserin and/or (b) counteracted potential strain differences in 5-$HT_{2A/2C}$ receptor functioning.

If, indeed, 5-HT_2 receptors were responsible for the strain difference in response to fluoxetine, then a combination of fluoxetine and ketanserin should have produced a strain difference in lordosis inhibition. An additive effect between the two drugs was anticipated since fluoxetine has 5-HT_2 receptor antagonist properties (Pälvimäki et al., 2005). Based on fluoxetine's 5-HT_2 receptor antagonist activity and prior studies that ketanserin amplified effects of SSRIs (Boothman et al., 2006), it was surprising that the

combination of fluoxetine and ketanserin did not have an additive effect. Boothman et al. reported that the route of drug administration may be important when SSRIs and a 5-HT_{2C} antagonist were being used together (Boothman et al., 2006). For example, they found greater augmentation when the SSRI was combined with local infusion of a 5-HT_2 antagonist. Hence, it is possible that the failure to observe such augmentation in the current study was the result of the systemic application of both drugs. In addition, 5-HT_2 receptor antagonists may not be as effective in combination with fluoxetine as with other SSRIs (Boothman et al., 2006).

Therefore, the collective data are most consistent with the following model.

1. Fischer rats have more SERT in the DRN than do Sprague-Dawley so that fluoxetine's increase in extracellular 5-HT in the DRN would be smaller in Fischer.
2. 5-HT_{1A} autoreceptors on serotonergic neurons inhibit firing of the serotonergic neurons in the DRN.
3. An increase in the extracellular 5-HT would increase activation of 5-HT_{1A} autoreceptors on the serotonergic neurons in the DRN.
4. This would reduce the amount of extracellular 5-HT in the VMN.
5. Fluoxetine blocks SERT and if there are more SERT present, it would require more fluoxetine to saturate SERT.
6. Fischer females would, therefore, have less activation of 5-HT_{1A} autoreceptors following fluoxetine.

7. Fischer rats would then have more 5-HT released in the VMN and therefore show greater lordosis inhibition than Sprague-Dawley rats.
8. Fischer rats would then have more 5-HT released in the VMN and therefore show greater lordosis inhibition than Sprague-Dawley.

In summary, activation of 5-HT_{1A} receptors is an important factor for lordosis behavior. The strain differences in SERT in the DRN could explain the importance of 5-HT_{1A} receptor activation and the sequence of events that can result in the decline in lordosis in Fischer rats. The observed strain differences to fluoxetine in intact, naturally cycling rats and ovariectomized rats and the effects of 8-OH-DPAT on lordosis in the two strains and the absence of such strain differences in response to ketanserin implicate strongly the role of 5-HT_{1A} receptors in fluoxetine-mediated lordosis inhibition. Ketanserin and fluoxetine were not additive in their effect on lordosis; their combination reflected primarily the lordosis inhibition with ketanserin alone. The modest strain differences in response to 5-HT_2 receptor antagonism cannot explain earlier finding of strain difference to fluoxetine. However, future studies with a more selective antagonist might be worthwhile. The individual difference in occurrence of SSRI-induced sexual dysfunction varies within the human female population. One possible candidate for these differences in sensitivity may be SERT, which plays a major role in reuptake of 5-HT and for which genetic polymorphisms exist in the human population. This could be one direction to explore that may help to understand the individual differences in sexual dysfunction in women who are treated with SSRIs.

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Education

2013: Ph.D in Molecular Biology
Texas Woman's University, Denton, Texas – 76204, USA

2005: Master of Science (M.Sc) in Microbiology, St. Francis Post Graduate College For Women, Hyderabad (Affiliated to Osmania University), Andhra Pradesh, INDIA

2002: Bachelor of Science (B.Sc) in Microbiology
St. Francis Degree College for Women, Hyderabad (Autonomous), (Osmania University), Andhra Pradesh, INDIA

Skills and Techniques

Animal Behavior

- Rat microsurgery, ovariectomy, behavioral testing/scoring and analysis and handling and injection of rats.
- Intracranial implant in rats

Neurobiology

- Brain slicing, perfusion and brain slice staining (histology) and rat estrous cycle assessment.

Molecular techniques

- DNA isolation and purification, gel electrophoresis, SDS-PAGE, cloning, RT-PCR and western blotting, ELISA and basic microscopic techniques and staining

Analysis skills

- Experimental study design, statistical analysis of data using SPSS, Delta graph software and office software

Research Experience

Graduate Research Assistant, Laboratory of Dr. Lynda Uphouse, Department of Biology, Texas Woman's University, Denton, Texas (June 2013-Aug 2013)

Conceptualized and conducted behavioral studies in rats, data collection from experiments followed by data analysis using statistical tools such as SPSS. Prepared manuscripts, poster preparation and presentation at national conferences and at Texas Woman's University symposia, trained undergraduate students in laboratory experimental techniques.

Graduate Research Assistant, Office of Technology, Texas Woman's University, Denton, Texas (Jan 2013- May 2013)

Recruited participants for the study designed to determine optimal ranges for visual angle, viewing angle and brightness of projector screen used in classroom for presenting static and high text-images. Assisted with design and conducting classroom studies, related data collection and analysis and assisted with manuscript writing.

Graduate Research Assistant, Laboratory of Dr. Lynda Uphouse, Department of Biology, Texas Woman's University, Denton, Texas (2009-2012)

Conceptualized, designed and conducted behavioral studies in rats to study the strain differences in response to acute treatment with Prozac®, data collection from experiments, data analysis using statistical tools such as SPSS. Manuscript preparation, poster preparation and presentation at national conferences and at Texas Woman's University symposia, trained undergraduate students in laboratory.

Teaching experience

Graduate Teaching Assistant, Department of Biology, Texas Woman's University, Denton, Texas (2007-2009)

Instructed undergraduate microbiology laboratory, administered exams, quizzes, graded exams and reported class progress to supervisor. Worked towards the objective of training students to work independently and as a team and directed them to accomplish the course objectives.

Instructor, Department of Microbiology, St. Francis Degree College for Women, Hyderabad, Andhra Pradesh, INDIA (2006-2007)

Instructed lecture and laboratory classes for undergraduate Microbiology course, demonstrated and trained students to conduct experiments, helped students to work towards the course goals and reported class progress to supervisor.

Instructor, Department of Microbiology, NMR College, Hyderabad, Andhra Pradesh, INDIA (2005-2006)

Instructed lecture and laboratory classes for undergraduate Microbiology course, prepared and set up laboratory prior to conducting experiments, demonstrated and trained students to conduct experiments, helped students to work towards the course goals and reported class progress to supervisor.

Research Projects

- Effect of 5-HT_2 receptor antagonist, ketanserin, on sexual behavior in Fischer and Sprague-Dawley strains
- Strain differences in response to fluoxetine induced sexual dysfunction
- RU486, a progesterone receptor antagonist, reduces progesterone's protection against the effects of mild restraint
- Medroxyprogesterone, a synthetic progesterone, prevents lordosis inhibition after mild restraint

- Effect of finasteride on progesterone-induced resistance to restraint stress
- 8-OH-DPAT modulation of fluoxetine-induced anorexia in ovariectomized rats with and without hormone priming

Research Publications

Miryala,C.S.J., Hiegel,C. and Uphouse,L. 2013. *Comparison of female Fischer and Sprague-Dawley rats in the response to ketanserin.* Submitted to Pharmacol. Biochem. Behav.

Miryala, C.S.J., Hiegel, C. and Uphouse, L. 2012. *Sprague-Dawley and Fischer female rats differ in acute effects of fluoxetine on sexual behavior*. J. Sex. Med. doi: 10.1111/j.1743-6109.2012.02981.x

Adams, S., **Miryala, C.S.J**., Hassell, J., Uphouse, L. 2012. *RU486 blocks effects of allopregnanolone on the response to restraint stress*. Pharmacol. Biochem. Behav.\., doi:10.1016/j.pbb.2012.09.024

Miryala, C.S.J., Hassell, J, Adams, S., Hiegel, C., Uzor, N. and Uphouse, L. 2011. *Mechanisms responsible for progesterone's protection against lordosis inhibiting effects of restraint. II. Role of Progesterone metabolites*. Horm. Behav., 60: 219-224. doi:10.1016/j.yhbeh.2011.05.006

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Miryala, C.S.J., N., Maswood and Uphouse, L. 2011. *Fluoxetine prevents 8-OH-DPAT-induced hyperphagia in Fischer inbred rats*. Pharmacol. Biochem. Behavior, 98:311-31.5 doi:10.1016/j.pbb.2011.01.014

Research Presentations

- **Annual Student Creative Arts and Research Symposium at Texas Woman's University, Denton, Texas.**

2013: **Chandra Suma Johnson Miryala**, Cindy Hiegel and Lynda Uphouse. Do Fischer and Sprague-Dawley females differ in their response to 5-HT_2 receptor antagonist?
2012: **Chandra Suma Johnson Miryala,** Cindy Hiegel, Sarah Adams, and Lynda Uphouse. Fischer and Sprague-Dawley female rats show different sensitivities to serotonin 2 receptor blockages
2012: Aminata Diaby, Cindy Hiegel, **Chandra Suma Johnson Miryala** and Lynda Uphouse. Estradiol benzoate, alone, is sufficient for induction of lordosis behavior
2012: Sarah Adams, James Hassell, **Chandra Suma Johnson Miryala**, Cindy Hiegel, and Lynda Uphouse. RU486 blocks protective effects of allopregnanolone on the response to restrain
2012: Sarah Ndedi, **Chandra Suma Johnson Miryala** and Lynda Uphouse. Fluoxetine reduces the female's active investigation of a male incentive
2011: **Chandra Suma Johnson Miryala**, Cindy Hiegel, James Hassell, and Lynda Uphouse. Strain differences in antidepressant-induced sexual dysfunction

2010: **C.S. Miryala**, J. Hassell, C. Hiegel, L. Uphouse. Finasteride fails to prevent progesterone's protection against mild restraint
2009: **Miryala, C.S.J**., N., Maswood and Uphouse, L. Does 8-OH-DPAT attenuate fluoxetine induced anorexia.

- **Federation of North Texas Area Universities, Graduate Student Research Symposium**

2013: **Chandra Suma Johnson Miryala**, Cindy Hiegel and Lynda Uphouse. Do Fischer and Sprague-Dawley females differ in their response to 5-HT_2 receptor antagonist?
2012: **Chandra Suma Johnson Miryala,** Cindy Hiegel, Sarah Adams, and Lynda Uphouse. Fischer and Sprague-Dawley female rats show different sensitivities to serotonin 2 receptor blockages
2011: **Chandra Suma Johnson Miryala**, Cindy Hiegel, James Hassell, and Lynda Uphouse. Strain differences in antidepressant-induced sexual dysfunction
2010: **C.S. Miryala**, J. Hassell, C. Hiegel, L. Uphouse. Finasteride fails to prevent progesterone's protection against mild restraint

- **Society for Neuroscience Presentations**

2011: **C.S.J Miryala**, James Hassell, Cindy Hiegel, and Lynda Uphouse. Female Fischer and Sprague-Dawley rats differ in fluoxetine-induced sexual dysfunction, annual Meeting of the Society for Neuroscience, Washington D.C., November 2011
2010: L.L. Uphouse, J.E. Hassell, **C.S.J. Miryala** and C. Hiegel. Redundant mechanisms involved in progesterone's protection against restraint-induced inhibition of lordosis behavior, Annual Meeting of the Society for Neuroscience, San Diego, November 2010

Professional Membership

Member of Society for Neurosciences

Membership in Honor Societies

Phi Kappa Phi

Scholarships

- Former Student Association scholarship for the period 2011-2012
- TWU General Scholarship for the period 2010-2011

Awards

2011, 2012 and 2013: Chancellor's Student Research Scholar award

2013: First prize for poster presentation titled "Do Fischer and Sprague-Dawley females differ in their response to 5-HT_2 receptor antagonist?" at the Federation of North Texas Area Universities Graduate Student Research Symposium

TWU Services

2008-2011 Assisted with the conference 'Expanding Your Horizons' by American Association of University Women
2009 Judge at TWU Science Fair

References

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